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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

ISSN 0706-652X / CJFSDX 52(8) 1597–1820 (1995)

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Volume 52, Number 8, August 1995

Canadian Journal of Fisheries and Aquatic Sciences

Published by the National Research Council of Canada

Volume 52, numéro 8, août 1995

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Publié par le Conseil national de recherches du Canada

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Mercury methylation and demethylation in aerobic surface waters

Tuula Matilainen and Matti Verta

Abstract: Potential rates of methylmercury formation and demethylation were studied in runoff waters, sediment trap material, and aerobic water layers of five small forest lakes by radioisotope methods. In addition, the roles of microbes and particulate matter in methylation and demethylation processes were examined. Methylation rates were low ($\leq 0.12\% \cdot d^{-1}$) in all sites studied. Water pH (4.9–6.9) had no clear effect on methylmercury formation. Methylation took place in filtered water (nominal pore size 0.2 μm) and in formaldehyde-treated or autoclaved samples, but was strongly suppressed by both dissolved organic matter (color) and particulate matter. Demethylation rates in surface waters ($\leq 13.2\% \cdot d^{-1}$) were decreased by low temperatures and the process was blocked by sterile filtration, formaldehyde treatment, and autoclaving. The results indicate that mercury methylation in aerobic humic waters is an accidental process caused by bacterial exoenzymes or other dissolved compounds. Methylmercury demethylation was clearly microbial in nature.

Résumé : Au moyen de radio-isotopes, nous avons évalué les valeurs possibles du taux de formation de méthylmercure et de déméthylation dans les eaux de ruissellement, dans les matériaux des pièges à sédiments et dans les couches d'eau aérobies de cinq petits lacs de forêt. En outre, nous avons examiné l'influence des microorganismes et des particules sur la méthylation et la déméthylation. La vitesse de méthylation était faible ($\leq 0{,}12\% \cdot jour^{-1}$) dans tous les sites étudiés. Par ailleurs, le pH de l'eau (4,9–6,9) n'a pas eu d'effet clairement identifiable sur la formation de méthylmercure. La méthylation s'observait dans l'eau filtrée (grandeur de pore nominale : 0,2 μm) et dans les échantillons traités au formaldéhyde ou autoclavés, mais elle était fortement inhibée dans les matières organique en solution (couleur) et dans les particules. La vitesse de déméthylation dans les eaux superficielles ($\leq 13.2\% \cdot jour^{-1}$) diminuait à basse température et le processus a été stoppé par la stérilisation par filtration, par le traitement au formaldéhyde et par l'autoclavage. Les résultats indiquent que la méthylation du mercure dans les eaux aérobies riches en humus est un processus accidentel causé par des exoenzymes bactériennes ou par d'autres composés en solution. La déméthylation du méthylmercure est manifestement un processus de nature microbienne.

[Traduit par la Rédaction]

Introduction

Methylmercury (MeHg) formation has been reported to occur in different sites of the aquatic ecosystem. Higher methylation rates have been measured in sediments than in the water phase (Callister and Winfrey 1986; Korthals and Winfrey 1987). Low pH and elevated mercury concentrations in aerobic water can stimulate net methylmercury production (Xun et al. 1987; Winfrey and Rudd 1990; Miskimmin et al. 1992). Methylation in littoral sediments increases with increasing temperature (Korthals and Winfrey 1987; Matilainen et al. 1991; Ramlal et al. 1993), as does the ratio of methylation/demethylation in epilimnetic water (Ramlal et al. 1993). Increased DOC (dissolved organic carbon) concentration and the addition of fresh sediment trap material to lake water decrease methylation rates; the demethylation process shows the opposite trend for pH and DOC (Miskimmin et al. 1992).

Methylation rates (Olson and Cooper 1976; Rudd et al. 1983; Compeau and Bartha 1984; Matilainen et al. 1991) and MeHg concentrations (Bloom et al. 1991; Mason et al. 1993; Parkman et al. 1994; Verta et al. 1994) are higher in anaerobic than aerobic conditions. However, there are at least three reasons that make aerobic surface waters worth investigating. (*i*) Atmospherically derived mercury, found in lake biota and sediments, has no other route to enter the lake than the runoff waters and the water surface of the lake basin. Many chemical, physical, and biological

Received July 5, 1994. Accepted March 2, 1995.
J12464

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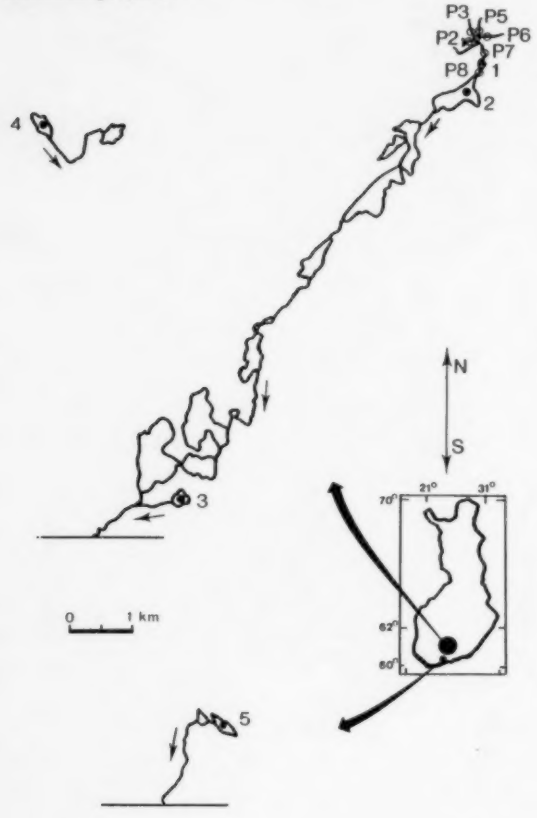
Fig. 1. Location of the study lakes (1–5) and runoffs (P2, P3, P5, P6). 1, Keskinen Hakojärvi (KEHA) and its inlet (P7) and outlet (P8); 2, Hakojärvi (HAKO); 3, Iso Valkjärvi (IVA); 4, Valkea Kotinen (VALKO); 5, Iso Lehmälampi (ILE).



transformations of mercury species take place in aerobic waters. (*ii*) The volume of the aerobic epilimnion is usually far greater than that of the anaerobic hypolimnion, and during stagnation there may be little or no diffusive transfer of MeHg. (*iii*) The food web, in which MeHg is finally concentrated to fish, is active in the aerobic water environment.

In the present study we examined the potential for MeHg formation and degradation in runoff waters, in the aerobic water columns of seepage and drainage lakes, and in sediment trap material. We also performed several treatment experiments to test the role of microbial activity and particulate matter in methylation and demethylation processes in aerobic humic waters.

Materials and methods

Study sites and sampling

The five lakes chosen for this study were located in southern Finland, in an area far from local Hg sources (Fig. 1). The Hg source in the catchments is atmospheric and during 1991–1992 the estimated wet depositions of total Hg and MeHg were 2.6 and 0.07 $g \cdot km^{-2} \cdot year^{-1}$, respectively (Verta et al. 1994). The vegetation of the lake catchments was primarily Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L.) over morainic soil and was partially covered with shallow peatland (usually <1 m). Most of the peatland was drained for forestry in the late 1960s. Extensive forest management, including clear-cutting and forest fertilization, has been performed in most of the catchments during the last 30 years.

Data presented in this paper were collected during 1990 and 1991. In 1990, the research was concerned with the temporal fluctuation of methylation and demethylation in different sites. These processes were more closely examined during 1991.

Water samples were taken from four small runoffs (P2, P3, P5, P6) entering two polyhumic drainage lakes downstream (Keskinen Hakojärvi (KEHA) and Hakojärvi (HAKO)) from the inlet and outlet of Lake KEHA (P7 and P8, respectively), and from three seepage lakes (Iso Valkjärvi (IVA, oligo-mesohumic), Valkea Kotinen (VALKO, humic), and Iso Lehmälampi (ILE, oligohumic)) during the ice-free season in 1990 (Fig. 1; Table 1). Lake KEHA was dammed by beavers in fall 1989 resulting in flooding and the enlargement of the area of the lake surface by some 30% (Verta et al. 1994). Lake IVA was divided into two subbasins by a plastic curtain in April 1991, and half was limed. The samples were taken with a Ruttner sampler or directly into 1-L borosilicate glass bottles from a depth of 1 m at the lake or runoffs, respectively. Several depths were sampled in aerobic water columns of lakes HAKO and ILE. The samples were kept cool and dark until processing.

In lakes HAKO, VALKO, IVA, and ILE, five parallel sediment traps (5 cm diameter, 50 cm height) were placed 1 m above the sediment surface at the deepest point of the lake. In Lake HAKO, sediment traps were also placed at 5 m depth. The traps were sampled at 1- to 3-month intervals by drawing the suspension from the bottom of the collectors into 100-mL plastic syringes (Once, Asik ApS, Denmark). In cases of anoxia, air contact was avoided by rinsing the syringe with anoxic trap water before the final sampling. After filling, the syringe was capped tightly. In the laboratory the anoxic samples were handled under nitrogen gas.

Determination of methylation and demethylation

The potential rates of methylation and demethylation were determined by using the modified radioisotope methods of Furutani and Rudd (1980) and Ramlal et al. (1986), respectively (see also Matilainen et al. 1991). Laboratory incubations were carried out within 20 h of sampling. The Hg methylation activity of the duplicate samples and one acid-killed blank (4 mL of 4 M HCl) was determined by incubating 100 mL of water or trap material with 2.5 μg Hg (22–67 kBq) as $^{203}HgCl_2$ (Amersham). For MeHg demethylation incubations, duplicate samples of 50 mL and a blank (2 mL of 4 M HCl) were incubated with 0.33 μg Hg (0.92 kBq) as $^{14}CH_3HgI$ (Amersham). The demethylation apparatus consisted of a 100-mL infusion bottle, a Teflon-covered magnetic rod, a gas-proof rubber stopper, and a threadable plastic cap holder. The samples were incubated in the dark and at an in situ temperature (±2°C) for 24 h.

Table 1. General characteristics and epilimnetic water quality of the lakes during autumn overturn in 1990.

	KEHA[a,b]	HAKO[a]	VALKO[c]	IVA[d]	ILE[c]
Max depth, m	6	19	7	8	7
Lake area, ha	1.3	17	4	4	6
Drainage area, ha	1300	200	70	17	30
pH range	5.6–6.5	5.5–7.2	5.2–5.8	5.1–5.7	4.8–5.1
pH	6.1	6.4	5.6	5.5	4.9
Alkalinity, mmol·L^{-1}	0.25	0.08	0	−0.02	−0.04
Conductivity, mS·m^{-1}	5.3	3.8	3.1	1.4	2.7
Color, mg Pt·L^{-1}	270	140	80	70	15
COD_{Mn}, mg·L^{-1}	30	14	11	9	3
Total N, μg·L^{-1}	940	540	420	510	510
Total P, μg·L^{-1}	160	27	14	20	10
Na, mg·L^{-1}	2.2	1.6	1.4	0.4	1.1
K, mg·L^{-1}	1.0	0.8	0.4	0.5	0.2
Ca, mg·L^{-1}	5.1	3.6	2.3	0.9	1.4
Mg, mg·L^{-1}	1.3	1.1	0.8	0.2	0.4
Al, μg·L^{-1}	383	250	152	52	180
Fe, mg·L^{-1}	1.86	1.20	0.25	0.28	0.26
Mn, μg·L^{-1}	66	160	29	36	36
SO_4, mg·L^{-1}	5.2	7.3	12.0	2.9	6.3
Cl, mg·L^{-1}	0.8	1.2	1.4	0.8	1.6
Hg,[e] ng·L^{-1}	7.2	3.2	na	1.3	na
MeHg,[e] ng·L^{-1}	2.3	0.40	na	0.11	na

Note: COD, chemical oxygen demand; na, not analyzed.
[a]Drainage, outlet.
[b]18 July 1990.
[c]Seepage, outlet.
[d]Seepage, outlet periodically.
[e]Data from Verta et al. (1994).

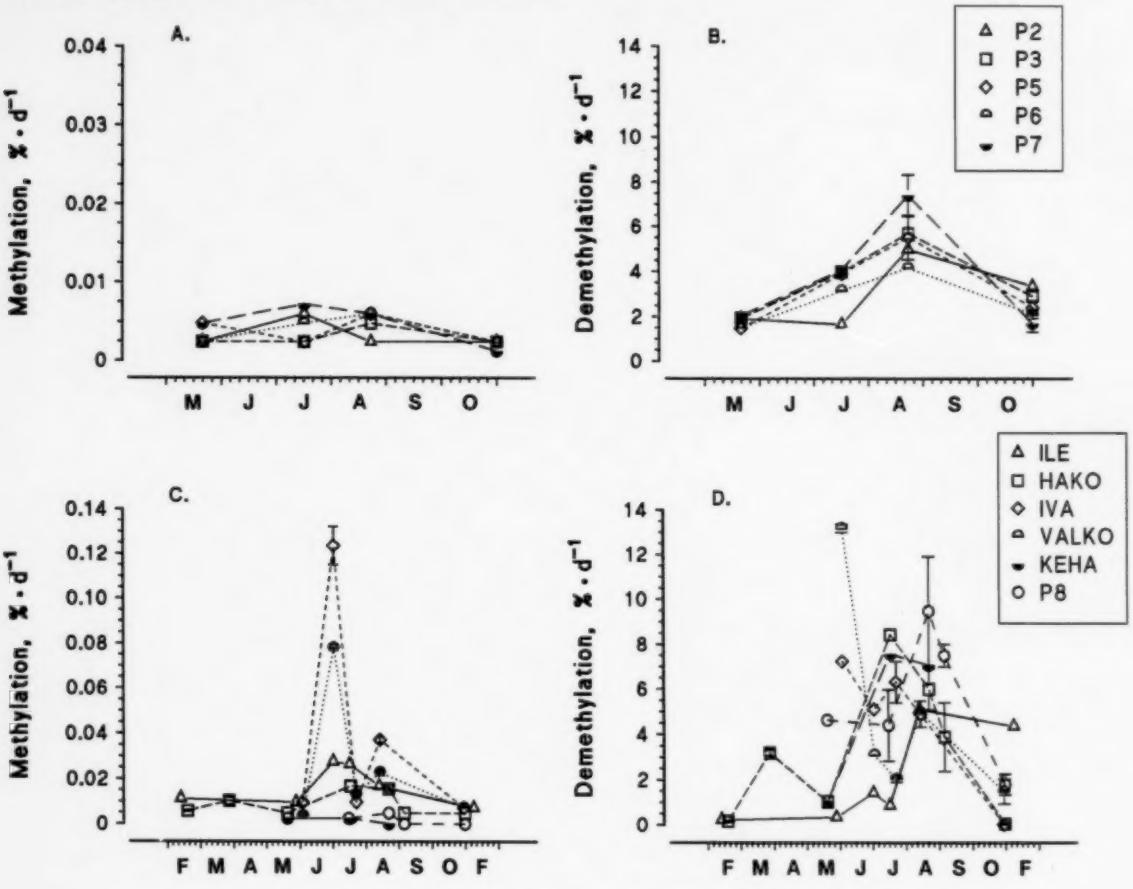
The MeHg formed during the incubation of the methylation assays was extracted after termination of the assay with 4 mL of 4 M HCl. The reagents (p.a.) were from Merck. The extraction procedure consisted of the following steps: (*i*) additions of 4 mL of 0.5 M $CuSO_4$ and 10 mL of 3 M NaBr in 2 M H_2SO_4, vigorous shaking after each addition for 1–2 min; (*ii*) addition of 20 or 25 mL of toluene, shaking for 5 min; (*iii*) removal most of the aqueous phase; (*iv*) centrifugation (1600 × *g*, Hettich Rotixa) for 10 min; (*v*) drying of the toluene extract with anhydrous Na_2SO_4; (*vi*) shaking of 10 mL of the dried toluene extract with 5 mL of 0.0025 M $Na_2S_2O_3$ in 20% ethanol in a separatory funnel for 5 min; (*vii*) shaking of an aliquot of 4 mL from the aqueous (lower) phase with 1 mL of benzene in a test tube for 2 min; (*viii*) pipetting of 0.5 mL of benzene extract into the vial containing 10 mL of PCS (Amersham) (Furutani and Rudd 1980); (*ix*) measurement of the activity with a liquid scintillation counter (Rackbeta, LKB-Wallac, Finland). The extraction efficiency, which was tested routinely with $CH_3{}^{203}HgCl$ (Amersham) in acidified samples, varied from 86 to 95% and the data were corrected accordingly. The potential methylation rate is expressed as a percentage of the added [$^{203}HgCl_2$]-Hg methylated per day in the sample volume of 100 mL.

The products of $^{14}CH_3HgI$ demethylation ($^{14}CO_2$ and $^{14}CH_4$) were gassed with oxygen from acidified samples into a CO_2 trapping cocktail for 1 h. The gas was passed through a tube furnace (450°C, Thermolyne Corp., type 21100) to convert $^{14}CH_4$ to $^{14}CO_2$. The quartz tubes (internal diameter 4.0 mm, wall 1.0 mm; Heralux) inside the furnace were packed with CuO granules (Ramlal et al. 1986). Six samples were gassed simultaneously on a multipoint magnetic stirrer (Variomag HP6). Oxygen flow was connected into and out of the gas space of each sample bottle through the stopper by silicon tubing and injection needles (Bovi-Vet, 2.10 × 80 mm). The total volume of both the trapping and scintillation cocktails was 8 mL (Lumasorb II and Carboluma; Lumac/3 M B.V., The Netherlands). The radioactivity was measured as above. Lumasorb II collected all the volatile $^{14}CO_2$ and there was no need for the second trap vial. The efficiency of stripping tested with $NaH^{14}CO_3$ varied from 75 to 95%. The potential rate of demethylation is expressed as a percentage of added [$^{14}CH_3HgI$]-Hg demethylated per day in the sample volume of 50 mL.

Laboratory experiments

The role of microbes and particulate matter in MeHg formation and degradation in aerobic waters was examined in the laboratory using different treatments. In the first experiment on 4 September 1991, water samples were taken from pelagic and (or) littoral sites of lakes KEHA (0.1 and 1 m), and IVA (0.1 m) and from runoff P3. Water and trap samples

Fig. 2. Hg methylation and demethylation during the study period. (A and B) Runoff waters and inlet of Lake KEHA, P7. (C and D) Lake waters, 1 m and Lake KEHA outlet (P8).



of Lake IVA were examined on 1 October 1991 (experiment 2). The rates of methylation and demethylation were measured in samples treated with HCl (final concentration 0.15 or 0.3 M) or with formaldehyde (final concentration 3.2%; Merck, 37%, stabilized with 10% methanol) or in samples filtered through GF/C (Whatman, retention 1.2 μm) and 0.2 μm (sterile SM 16534, Sartorius) filters.

For the third experiment, in February 1992, water was taken from the outlet of Lake HAKO in October 1991 and stored at 4°C in the dark until examination. In February, aliquots of water were filtered through either GF/C (1.2 μm), GF/F (0.7 μm), or 0.2-μm (sterile SM 16534, Sartorius) filters then subjected to treatment with HCl or formaldehyde, or were autoclaved (1 atm, (1 atm = 101.325 kPa) 120°C, 15 min). A control of no treatment aside from filtering was also done.

To determine the radioactivity retained in particles larger than 0.2 μm during incubation of the methylation assays, a 5-mL aliquot was taken from the acid-terminated samples (before extraction of MeHg). The radioactivity of 40 μL of filtered (through a 0.2 μm filter) and unfiltered samples was measured in 10 mL of PCS and 0.5 mL benzene with a liquid scintillation counter. The samples studied were taken on 1 October 1991, and comprised water and sediment trap material from Lake IVA and water from P6, P7, and P8.

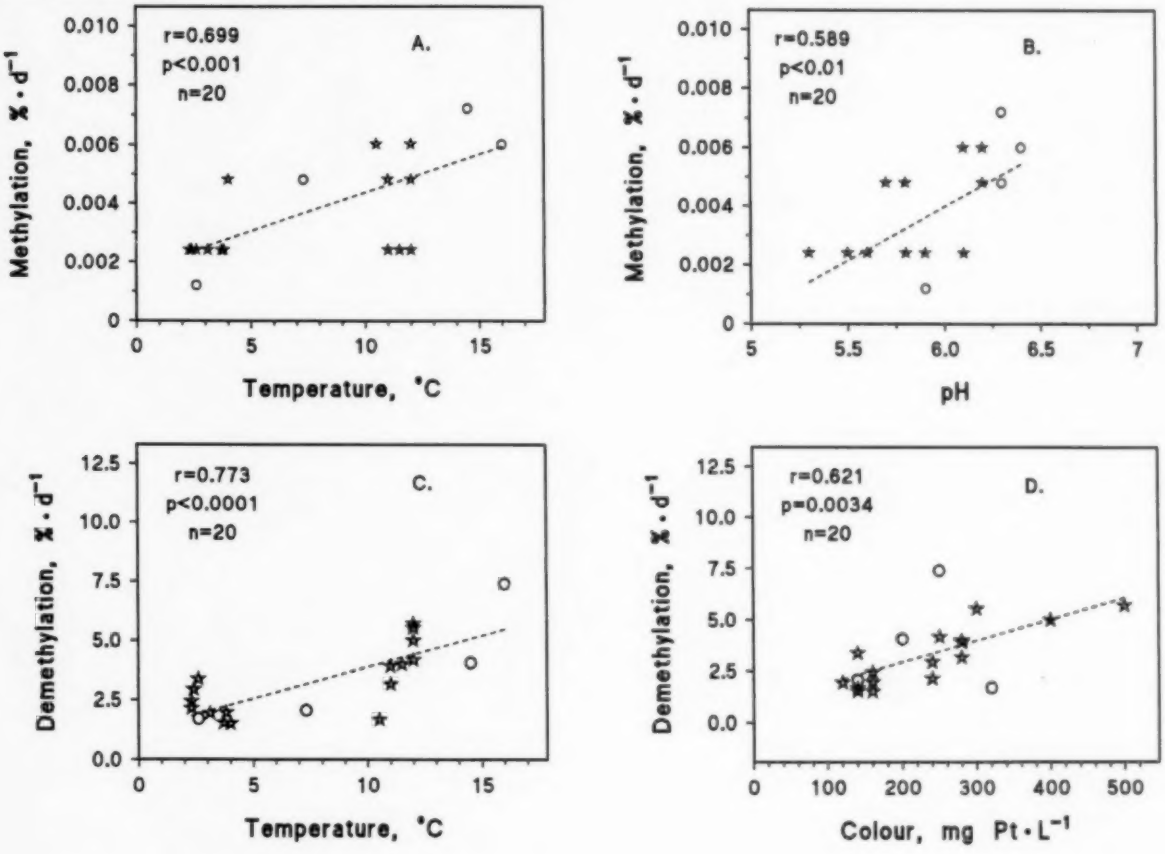
Results

Runoff waters (P2–P7)

The rate of methylation was extremely low in runoff waters (range 0.0012–0.0072%·d^{-1}, n = 20), and no clear peaks were detected between May and October (Fig. 2*a*). The waters were brown, with 120–500 mg Pt·L^{-1}, indicating the presence of dissolved humic compounds. Methylation rates, which were lowest in October at all sites, had significant positive correlations with water temperature and pH (Figs. 3*a* and 3*b*).

Demethylation in runoff waters showed a maximum in August at all stations (Fig. 2*b*) and ranged from 1.5 to 7.4%·d^{-1}. Demethylation had significant positive correlations with water temperature and color (Figs. 3*c* and 3*d*). The methylation/demethylation ratio (M/D), which roughly

Fig. 3. The relationship between Hg methylation and (A) temperature and (B) pH, and demethylation and (C) temperature and (D) color in runoff waters. ☆, P2–P6; ○, P7.



indicates the relative net MeHg formation, correlated negatively with water color (log transformation for M/D values: $r = -0.565$, $p < 0.01$, $n = 20$).

Lakes

Epilimnetic methylation of the lakes varied from 0.0024 to 0.124%·d^{-1}. The rates, which from winter to early June were as low as in runoff, peaked in June–August and decreased again in September–October (Fig. 2*c*). The exception was the polyhumic Lake KEHA, with very low methylation rates at all times measured. The data from Lake KEHA epilimnion were limited (three samples), but the same low rates were also measured in the lake outlet, P8 (Fig. 2*c*). Methylation (as log transformation; all lakes and seasons) had a strong negative correlation with water color (Fig. 4). A weak positive correlation was found between methylation (log) and water temperature in the whole data set (lakes and runoffs: $r = 0.484$, $p < 0.001$, $n = 51$).

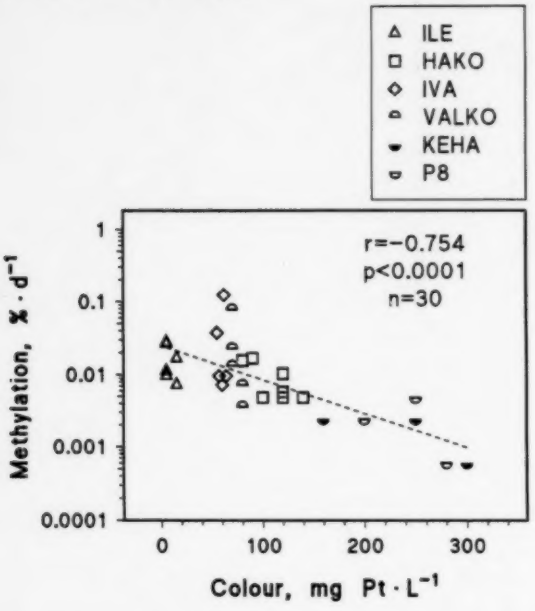
Demethylation in lake waters ranged from <0.1 to 13.2%·d^{-1}. The two seepage lakes VALKO and IVA showed high demethylation activity on the first sampling date, 4 June, 1 month after iceout (Fig. 2*d*). By that time the temperature at 1 m depth had already risen to 17–18°C, higher than that in the other lakes (11–13°C) at the same time. Similar to the data for runoff waters, demethylation correlated positively with temperature in the whole lake data ($r = 0.518$, $p < 0.01$, $n = 26$), and also during the ice-free season ($r = 0.445$, $p < 0.05$, $n = 23$).

Vertical profiles

In Lake ILE, with no thermal stratification, the vertical variation in methylation activity was low. Methylation rates varied from 0.005%·d^{-1} during the cold season to 0.028%·d^{-1} in summer. Demethylation rates were usually highest in the bottom layers. Very low demethylation rates were measured in winter and spring, but also in July when the temperature in the whole water column was high (18.1–19.6°C). Demethylation activity increased in late summer and fall (Fig. 2*d*).

Lake HAKO became stratified in late May; oxygen depletion developed in the hypolimnion during the summer and total anoxia occurred near the sediment surface in August–September (Fig. 5). Vertical variation in the methylation rates was minimal (Fig. 5) but rates were usually somewhat higher in the epilimnion (0.005–0.018%·d^{-1}) than in the hypolimnion (0.0025–0.01%·d^{-1}). Demethylation

Fig. 4. The relationship between Hg methylation (log transformation) and color in the study lakes (1 m depth) and Lake KEHA outlet (P8).



peaked in the warm 1-m samples, then decreased, and peaked again in the hypolimnion (Fig. 6).

Trap material

The methylation rate in the trap material was low (range 0.0006–0.0104%·d^{-1}) (Table 2). The highest rates were measured in July–August (mean 0.0071%·d^{-1}, n = 7) and the lowest in October–November (mean 0.0011%·d^{-1}, n = 5). In lakes ILE and HAKO (5 m) methylation activity in the water outside the collector was 2 to 16 times that in the trap material.

The rate of demethylation in the trap material was the same as or somewhat higher than in the water (Table 2; Figs. 2, 6). Demethylation rates varied from 3.84 to 12.85%·d^{-1} (mean 8.35%·d^{-1}, n = 7) in the summer months, and from 0.57 to 4.36%·d^{-1} (mean 1.42%·d^{-1}, n = 5) in October. In October the 15-m sample from Lake HAKO showed more efficient demethylation activity than the 5-m sample (4.36 and 0.62%·d^{-1}, respectively). The organic matter content (as loss of ignition) in the trap material reflected the content in the surface sediment (HAKO, 42–48%; ILE, 55–69%; VALKO, 63–87%; IVA, 64–87%).

Regression analysis

The regression models explained 56–75% of the methylation and 51–68% of the demethylation using only temperature and color as variables (Table 3). In all data sets temperature was the first variable to explain demethylation, whereas color was usually the main variable to explain methylation. This pattern remained the same irrespective of whether all data or only those from the ice-free period were included in the model runs. The model runs for the M/D ratio gave clearly lower R^2 values (from 0.32 to 0.56). pH was not selected as an explaining variable in any of the model runs. When demethylation was included as an independent variable to explain methylation it was not selected.

Table 2. The rates of methylation (M) and demethylation (D) in trap material (%·d^{-1}).

Lake	Date (in 1990)	M	D
HAKO (5 m)	Aug. 7	0.0056	11.44
	Oct. 30	0.0007	0.62
HAKO (15 m)	July 9	0.0101	8.17
	Aug. 7	0.0075	9.41
	Oct. 30	0.0019	4.36
VALKO (5 m)	Aug. 14	0.0104	3.84
	Oct. 29	0.0006	0.97
IVA (6 m)	Aug. 7	0.0050	6.63
	Oct. 29	0.0016	0.59
ILE (5 m)	July 2	0.0046	6.09
	Aug. 13	0.0064	12.85
	Nov. 17	0.0007	0.57

Impact of sterilization and particulate matter on methylation and demethylation

To explore the biotic or abiotic character of Hg methylation and MeHg demethylation in aerobic waters we examined these processes in the sterilized (0.2-μm filtration or autoclaving), or otherwise treated samples (HCl or formaldehyde, or GF/C filtration) (Fig. 7).

Hydrochloric acid did not totally prevent methylation at 0.15 M (Fig. 7*a*), but decreased it effectively at 0.3 M (Figs. 7*c*, 7*e*). Formaldehyde treatment (3.2%, v/v) increased methylation rates. Sterile filtration (0.2 μm), GF/C filtration, or autoclaving did not block methylation (Figs. 7*a*, 7*c*, 7*e*). Demethylation was completely prevented by the treatments with HCl, formaldehyde, sterile filtration, and autoclaving (Figs. 7*b*, 7*d*, 7*f*). The GF/C filtered samples behaved differently depending on the sample origin and time, indicating the different size (and density) distributions of microorganisms and other particles.

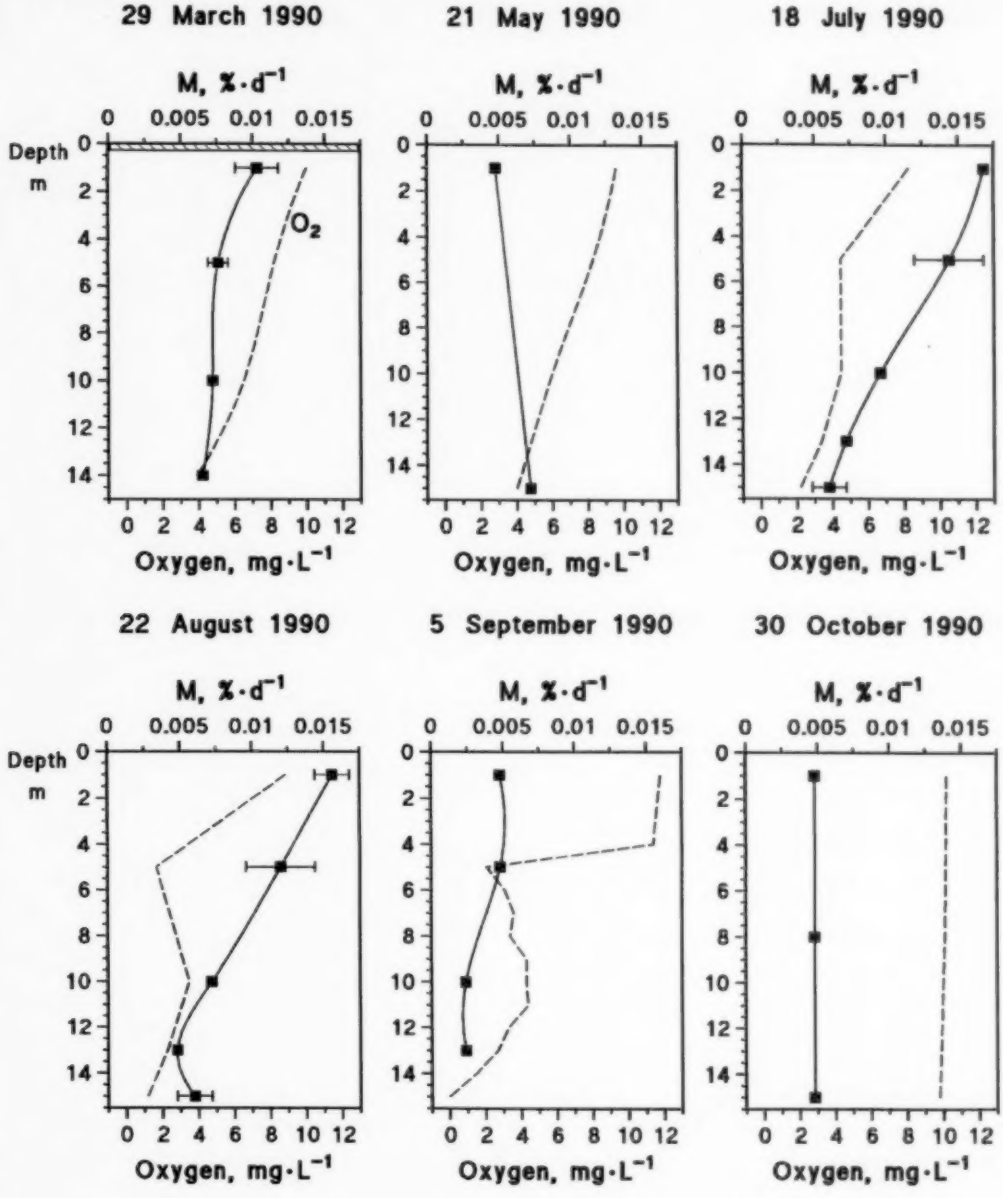
Formaldehyde and autoclaving treatments had no marked effect on the methylation rates in different filtrates (<0.2, <0.7, and <1.2 m) (Fig. 7*e*), but stopped demethylation completely (Fig. 7*f*). The rest of the demethylation activity in GF/C and GF/F filtrates disappeared when the samples were treated by HCl, formaldehyde, or autoclaving.

The amount of radioactivity bound to particulate matter (>0.2 μm) was negatively related to methylation rates in the unfiltered samples (Fig. 8). The correlation indicates that the availability of free Hg for methylation decreases with an increasing amount of particulate matter in water.

Discussion

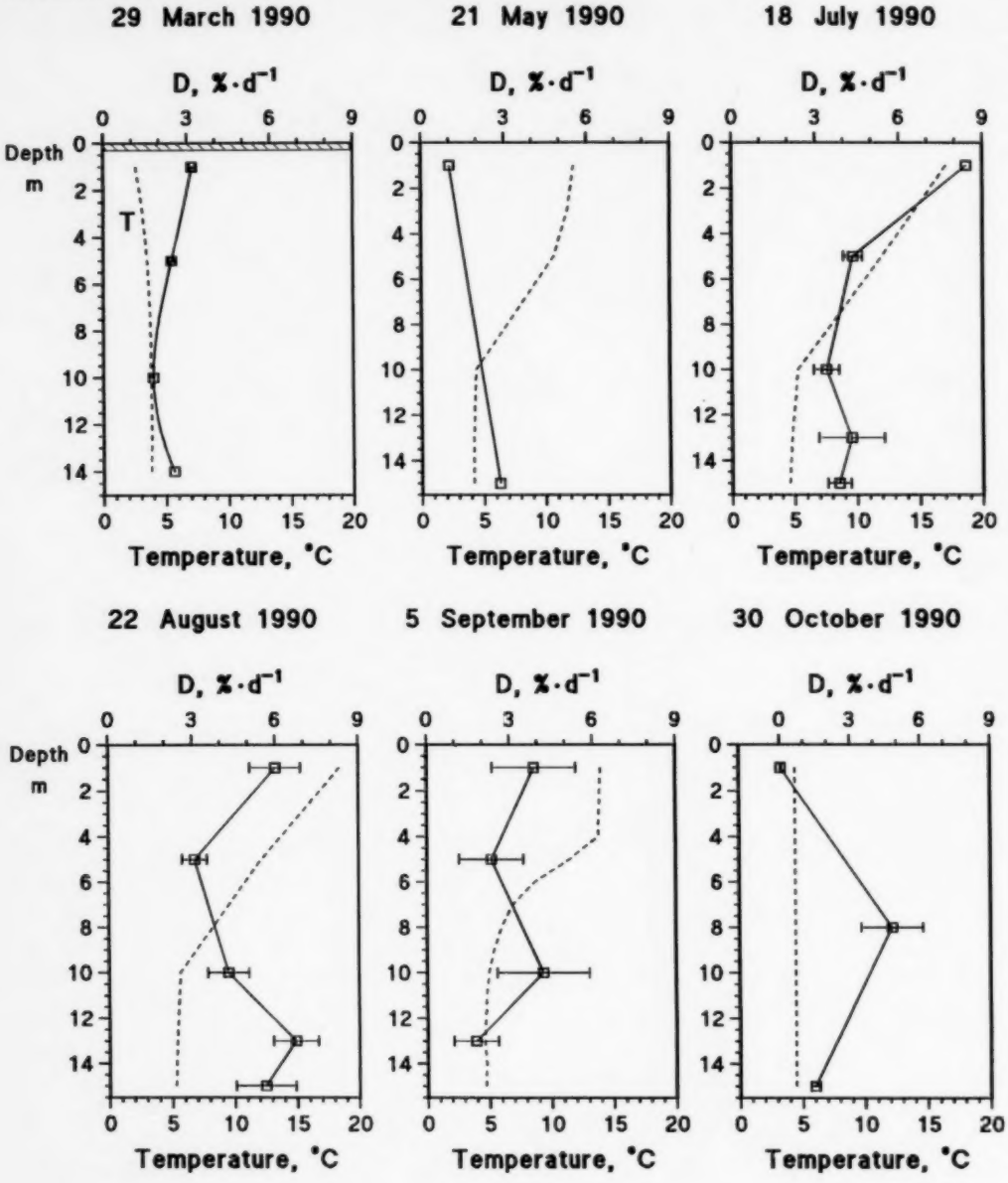
Of the variables studied, temperature and color were the major factors contributing to the potential net MeHg formation in surface waters. In our samples, color can be regarded as a measure of total and dissolved organic carbon,

Fig. 5. Hg methylation (■) and oxygen concentration (broken line) in Lake HAKO water column from 29 March to 30 October 1990.



because studies conducted in similar environments in Finland have shown a close relationship between these variables (Kortelainen 1993). The inhibitory effect of organic material on methylation and the M/D ratio was obvious in surface waters and was consistent with the finding of Miskimmin et al. (1992). On the other hand, demethylation increased with increasing organic material (color), which was also the case in laboratory experiments (Miskimmin et al. 1992). The rates of demethylation in our assays were two orders of magnitude higher than those of methylation (Fig. 2–5). Although ambient total Hg and MeHg concentrations were overwhelmed by the radioisotopes in the assays, and therefore the measured rates cannot be regarded as actual rates, it is possible that demethylation during the 24-h incubation affects methylation to some extent. However, it is not plausible that the DOC inhibition of methylation was caused by the stimulatory effect of DOC on demethylation, because the inhibitory effect on methylation was exponential (Fig. 4).

Fig. 6. MeHg demethylation (□) and temperature (broken line) in Lake HAKO water column from 29 March to 30 October 1990.



Moreover, demethylation was not selected by the models as an explaining variable of methylation, which is consistent with the results of Miskimmin et al. (1992).

Our original hypothesis, that a high concentration of MeHg in fish in a polyhumic lake is caused by increased methylation of Hg by humic substances (Verta 1984), was not valid as demonstrated by the inhibitory effect of DOC on MeHg formation. In several studies, however, MeHg has been found to correlate positively with humic substances in water, especially in runoff (Lee and Iverfeldt 1991; Westling 1991). High MeHg concentrations were also recorded in the runoffs of the present study in 1991 (Verta et al. 1994). It seems probable that in the MeHg cycle, humic substances act as MeHg carriers from catchments and not as active methylation agents.

High temperatures during summer clearly stimulated epilimnetic methylation and especially demethylation. The stimulative effect on demethylation was also seen in runoff

Table 3. Stepwise multiple regression of methylation and demethylation against water temperature and water color (as a measure of organic material).

		Log(methylation)		Demethylation	
		Variable	R^2 +/−	Variable	R^2 +/−
Runoff					
(n = 20)	Step 1	Temp.	0.46 +	Temp.	0.60 +
	Step 2	Color	0.13 −	Color	0.08 +
	Total		0.59		0.68
Lakes in summer					
(n = 26–28)	Step 1	Color	0.63 −	Temp.	0.38 +
	Step 2	Temp.	0.12 +	Color	0.13 +
	Total		0.75		0.51
All data					
(n = 49–51)	Step 1	Color	0.38 −	Temp.	0.46 +
	Step 2	Temp.	0.18 +	Color	0.10 +
	Total		0.56		0.56

Note: The table gives the variable entered at each step, the proportion of variation explained by each variable, and the cumulative percentage on explained variation.

waters (Fig. 2, Table 3). Although temperature was selected as the variable that best explained methylation in runoff (Table 3), an increase in methylation potential could barely be seen during the warm months (Fig. 2*a*). It appears that in our surface waters the inhibitory effect of DOC on methylation was stronger than the stimulatory effect of temperature (Figs. 2*a*, 2*c*).

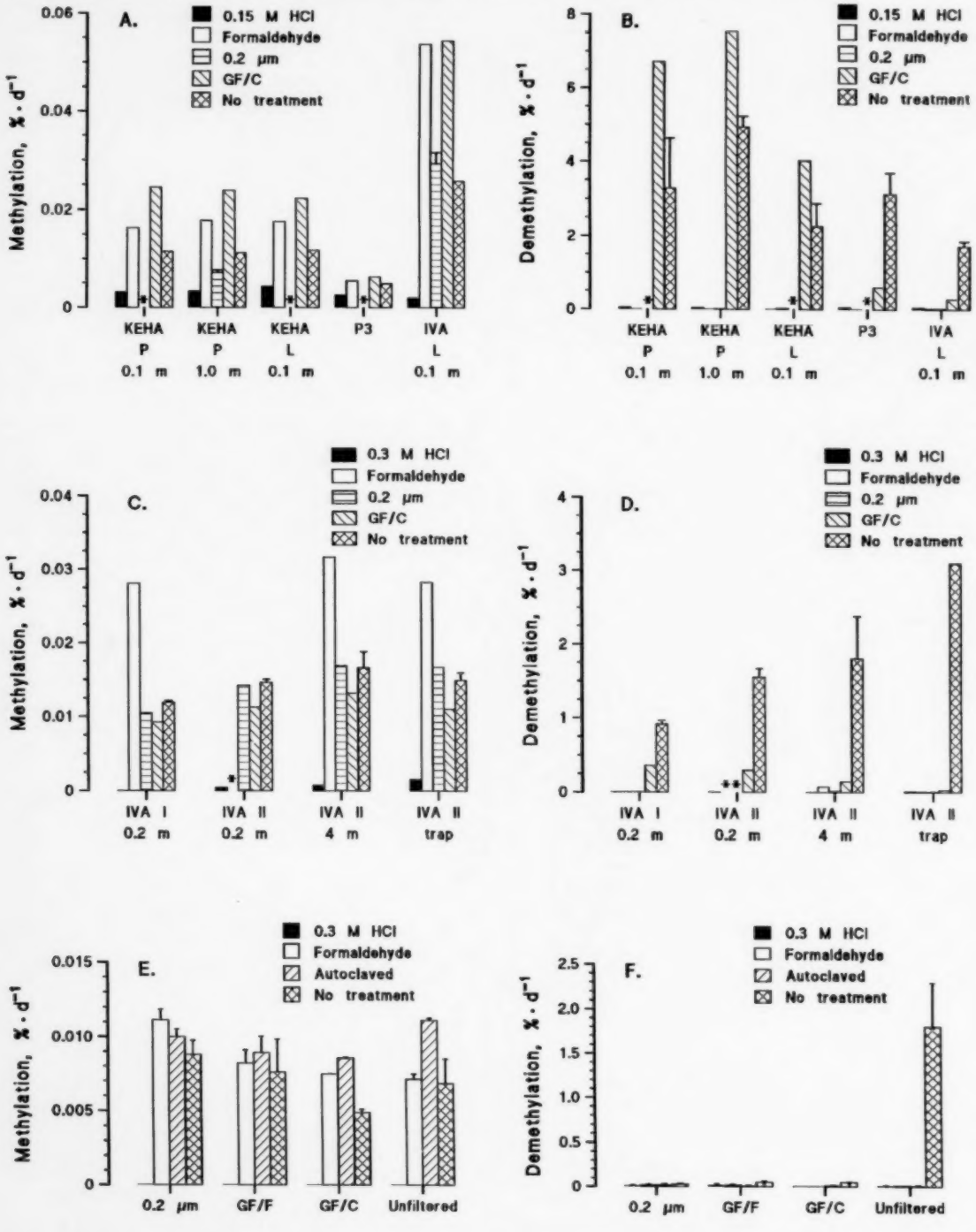
We found either a weak or no relationship between pH and MeHg formation in the surface waters studied. This finding is inconsistent with the results of the laboratory experiments by Miskimmin et al. (1992), who found that pH has a more important role than DOC in net MeHg formation. It is also inconsistent with the results of Xun et al. (1987) that net methylation is increased at low pH in laboratory experiments and in lake waters in Canada. Xun et al. (1987) did not report the total or dissolved organic carbon values of their lakes, however. The positive correlation between pH and methylation in runoff waters (Fig. 3*b*) may be an artefact owing to the limited data, low pH during spring flood, and an extremely low level of methylation.

Experiments on the elimination of bacterial activity (Fig. 7) clearly demonstrated that demethylation was purely microbial in nature. Sterile filtration, autoclaving, and the treatments with HCl and formaldehyde blocked the process completely. GF/C filtering decreased demethylation of the unfiltered samples from runoff P3 and Lake IVA by 60–100%. Alternatively, in the extremely humic Lake KEHA, GF/C filtering increased demethylation (Fig. 7*b*): the reasons for this higher demethylation activity may be the low primary production and high concentration of dissolved humic substances. In the unfiltered samples the bacterial populations were undoubtedly controlled by both microzooplankton (heterotrophic and mixotrophic flagellates and ciliates) and macrozooplankton (Jones 1992; Kuuppo-Leinikki and Salonen 1992; Salonen et al. 1992*a*, 1992*b*). Particles smaller than 1.2 μm including bacteria pass through the GF/C filter, but most of the zooplankton and algae are retained. Thus, in filtered samples, without the effect of grazing, the demethylating bacteria may have had an advantage in survival. The bacterial assemblages of both humic and nonhumic lakes have been reported to prefer photosynthetically produced DOC, which is released from phytoplankton under conditions of high primary production, whereas under periods of comparatively low primary production, heterotrophic bacteria in humic lakes utilize allochthonous humic substances as a substrate (Sundh and Bell 1992). These dissolved humic substances probably served as the alternative carbon source for the demethylating bacteria in the GF/C filtrates of Lake KEHA, whereas in the oligo-mesohumic Lake IVA the bacteria may have been attached to algal particles and retained by the filter, and the bacteria in the filtrates may have suffered from a lack of utilizable substrates. Demethylation in the GF/C filtrate of runoff P3 resembled that of Lake IVA (Figs. 7*b*, 7*d*); however, in this case the demethylators were attached more to the terrestial particulates than to algae.

Methylation was blocked by acid treatment whereas sterilization, GF/C filtration, autoclaving, or formaldehyde treatment did not prevent it (Figs. 7*a*, 7*c*, 7*e*), indicating that the presence of microbes was not the deciding factor for MeHg formation in aerobic waters. This is in agreement with the results of Parkman et al. (1994), who found that MeHg formation in a permanently stratified fiord took place during both oxidizing and reducing conditions and without bacteria. The authors suggested that methylation was an extracellular process probably enhanced by bacterial exoenzyme activity in environments where microbial decomposition of organic matter is important.

Formaldehyde treatment increased methylation, probably by one or several of the following processes: (*i*) elimination of microbial demethylation activity; (*ii*) the spontaneous

Fig. 7. Hg methylation and demethylation in aerobic samples with various treatments. (A and B) Water samples treated with HCl, formaldehyde, GF/C, or sterile filtering on 4 September 1991. For all sterile filtered samples and for control samples in demethylation assays, values are given as the mean ± 1 SD (n = 2); for all others, n = 1. *, not determined; P, pelagic; L, littoral. (C and D) Pelagic water and trap material of Lake IVA treated with HCl, formaldehyde, GF/C, or sterile filtering on 1 October 1991. For controls, values are given as the mean ± 1 SD (n = 2); for all others, n = 1. I, limed; II, control. (E and F) Lake HAKO outlet water in February 1992. Values are given as the mean ± 1 SD (n = 2).

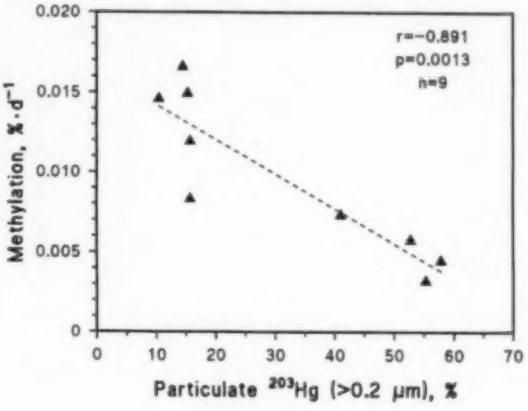


methylation by formaldehyde or methanol, which is used as a stabilizer in formaldehyde solutions; and (*iii*) the liberation of compounds capable of spontaneous methylation from bacterial or algal cells. The stimulation was lower in highly humic water (P3, lakes KEHA and HAKO) than in oligo-mesohumic water (Lake IVA) (Figs. 7*a*, 7*c*). This may be a reflection of the comparatively low density of phytoplankton cells and the lowering effect of dissolved humic substances on the overall methylation in humic waters. A study of the effects of poisons and preservatives on the rate of particle degradation and metal release (Lee and Fisher 1992) demonstrated that 24 mM formaldehyde effectively inhibited microbial activity and caused a rapid leaching of DOC compounds from phytoplankton cells, probably resulting from damage to the cell membrane. This phenomenon was probably responsible for the stimulation of methylation by formaldehyde in our experiments. Whether some of those DOC compounds were the methylating agents, e.g., methylcobalamine (Bertilsson and Neuhjar 1971; De Simone et al. 1973) or enzymes (Landner 1971), remains unknown.

Methylation was not stopped by GF/C filtration (Figs. 7*a*, 7*c*, 7*e*). It is difficult to explain why GF/C filtration increased methylation in the first experiment (Fig. 7*a*). However, it was seen in the demethylation assays that bacteria, including demethylators, passed through the GF/C filter. The high GF/C demethylation activity found in polyhumic Lake KEHA is an indication of high bacterial activity. Münster et al. (1992) found extracellular enzymes to be important regulators in detritus processing and substrate utilization in a polyhumic lake (300–600 mg $Pt \cdot L^{-1}$). The authors assumed that depolymerization of dissolved organic matter (extracellular substrate) can contribute to the availability and dynamics of utilizable dissolved organic matter. Those results applied to methylation and demethylation in the GF/C filtered waters of Lake KEHA and Lake IVA littoral (Figs. 7*a*, 7*b*), indicate that a dense bacterial population could have developed (or existed) without grazers. The availability of utilizable dissolved organic matter may have become a growth-limiting factor for heterotrophic bacteria and this resulted in increased excretion of extracellular enzymes for the cleaving of polymeric DOC compounds to easily utilizable monomers and oligomers. Thus, accidental MeHg formation by enhanced heterotrophic activity might have occurred in our GF/C filtered humic surface waters (Parkman et al. 1994). The reason for the different behavior of the methylation of GF/C filtrates in the experiments (Figs. 7*a*, 7*c*, 7*e*) may be the lower temperature and bacterial activity in October than in September.

Net MeHg formation in aerobic waters decreases when the amount of particle-bound Hg increases (Fig. 8). Many studies have demonstrated that Hg has a high affinity for organic matter (Andersson 1979; Rekolainen et al. 1986; Lindqvist et al. 1991). Korthals and Winfrey (1987) reported extremely low methylation and high demethylation activity in both aerobically incubated water and periphyton samples of an oligotrophic lake. Miskimmin (1989) demonstrated that the methylation rate of lake water samples was suppressed by added sediment trap material. In the present study too, the methylation rates were low and demethylation rates high in sediment trap suspension. Evidently, in addition to the suppression of methylation by dissolved humic substances, MeHg formation is strongly depressed by particulate organic matter, which effectively scavenges the Hg^{2+} from aerobic water.

Fig. 8. Hg methylation (as percentage of added radioactivity) of the untreated samples of water and trap material plotted against the percentage of the radioactivity bound to particles >0.2 μm during the 24-h incubation.



In conclusion, the Hg methylation potential was low in surface lake waters (1 m), aerobic water columns, sediment trap material, and runoff waters. Methylation took place in sterilized lake waters, and is probably an accidental reaction, possibly caused by enzymes or other compounds liberated during heterotrophic activity. Methylation was suppressed not only by dissolved humic substances but also by particulate organic matter, both of which can bind Hg in a form unavailable for methylation. The water pH (4.9–6.9) did not remarkably affect methylation in the waters studied. Methylation and demethylation increased with increasing temperature. The temperature dependence and the fact that sterilization blocked demethylation completely showed that the MeHg demethylation process was purely microbial in nature.

Acknowledgements

We thank A. Uusi-Rauva for advice on radioisotope questions and for the excellent laboratory facilities in the Equipment Center, University of Helsinki. Valuable comments on the manuscript were provided by M. Niemi. We also thank P. Tallberg and P. Porvari for their laboratory and field assistance. The work was supported by the Finnish Research Foundation for Natural Resources, the Maj and Tor Nessling Foundation, the National Board of Waters and the Environment, and the Academy of Finland.

References

Andersson, A. 1979. Mercury in soils. *In* The biochemistry of mercury in the environment. *Edited by* O. Nriagu. Elsevier, North-Holland Biomedical Press Amsterdam, The Netherlands. pp. 79–112.

Bertilsson, L., and H.Y. Neuhjar. 1971. Methylation of mercury compounds by methylcobalamin. Biochemistry, 10: 2805–2808.

Bloom, N.S., C.J. Watras, and J.P. Hurley. 1991. Impact of acidification on the methylmercury cycle of remote seepage lakes. Water Air Soil Pollut. 56: 477–491.

Callister, S.M., and M.R. Winfrey. 1986. Microbial methylation of mercury in Upper Wisconsin river sediments. Water Air Soil Pollut. 29: 453–465.

Compeau, G., and R. Bartha. 1984. Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. Appl. Environ. Microbiol. 48: 1203–1207.

De Simone, R.E., M.W. Penley, L. Charbonneau, S.G. Smith, J.M. Wood, H.A.O. Hill, J.M. Pratt, S. Ridsdale, and R.J.P. Williams. 1973. The kinetics and mechanism of cobalamin-dependent methyl and ethyl transfer to mercuric ion. Biochim. Biophys. Acta, 304: 851–863.

Furutani, A., and J.W.M. Rudd. 1980. Measurement of mercury methylation in lake water and sediment samples. Appl. Environ. Microbiol. 40: 770–776.

Jones, R.I. 1992. The influence of humic substances on lacustrine food chains. Hydrobiologia, 229: 73–91.

Kortelainen, P. 1993. Content of total organic carbon in Finnish lakes and its relationship to catchment characteristics. Can. J. Fish. Aquat. Sci. 50: 1477–1483.

Korthals, E.T., and M.R. Winfrey. 1987. Seasonal and spatial variations in mercury methylation and demethylation in an oligotrophic lake. Appl. Environ. Microbiol. 53: 2397–2404.

Kuuppo-Leinikki, P., and K. Salonen. 1992. Bacterioplankton in a small polyhumic lake with an anoxic hypolimnion. Hydrobiologia, 229: 159–168.

Landner, L. 1971. Biochemical model for biomethylation of mercury suggested from methylation study in vivo with *Neurospora crassa*. Nature (London), 230: 452–454.

Lee, B.-G., and N.S. Fisher. 1992. Degradation and elemental release rates from phytoplankton debris and their geochemical implications. Limnol. Oceanogr. 37: 1345–1360.

Lee Y.-H., and Å. Iverfeldt. 1991. Measurement of methylmercury and mercury in run-off, lake and rain waters. Water Air Soil Pollut. 56: 309–321.

Lindqvist, O. (*Editors*). 1991. Mercury in the Swedish environment. Recent research on causes, consequences and corrective methods. Water Air Soil Pollut. 55: 1–261.

Mason, R.P., W.F. Fitzgerald, J. Hurley, A.K. Hanson, Jr., P.L. Donaghay, and J.M. Sieburth. 1993. Mercury biogeochemical cycling in a stratified estuary. Limnol. Oceanogr. 38: 1227–1241.

Matilainen, T., M. Verta, M. Niemi, and A. Uusi-Rauva. 1991. Specific rates of net methylmercury production in lake sediments. Water Air Soil Pollut. 56: 595–605.

Miskimmin, B.M. 1989. The influence of dissolved organic carbon on methyl mercury production and sediment-water partitioning in precambrian Shield lakes. M.Sc. thesis, Department of Microbiology, University of Manitoba, Winnipeg, Manitoba.

Miskimmin, B.M., J.W.M. Rudd, and C.A. Kelly. 1992. Influence of dissolved organic carbon, pH, and microbial respiration rates on mercury methylation and demethylation in lake water. Can. J. Fish. Aquat. Sci. 49: 17–22.

Münster, U., P. Einiö, J. Nurminen, and J. Overbeck. 1992. Extracellular enzymes in a polyhumic lake: important regulators in detritus processing. Hydrobiologia, 229: 225–238.

Olson, B.H., and R.C. Cooper. 1976. Comparison of aerobic and anaerobic methylation of mercuric chloride by San Francisco Bay sediments. Water Res. 10: 113–116.

Parkman, H., P. Östlund, M.-O. Samuelsson, and Å. Iverfeldt. 1994. Methyl mercury in a permanently stratified fiord. *In* Mercury pollution: integration and synthesis. *Edited by* C.J. Watras and J.W. Huckabee. Lewis Publishers, Inc., Chelsea, Mich. pp. 107–118.

Ramlal, P.S., J.W.M. Rudd, and R.E. Hecky. 1986. Methods for measuring specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation. Appl. Environ. Microbiol. 51: 110–114.

Ramlal, P.S., C.A. Kelly, J.W.M. Rudd, and A. Furutani. 1993. Sites of methyl mercury production in remote Canadian Shield lakes. Can. J. Fish. Aquat. Sci. 50: 972–979.

Rekolainen, S., M. Verta, and A. Liehu. 1986. The effect of airborne mercury and peatland drainage on sediment mercury contents in some Finnish forest lakes. Vesientutkimuslaitoksen Julk. No. 65. pp. 11–20.

Rudd, J.W.M., M.A. Turner, A. Furutani, A.L. Swick, and B.E. Townsend. 1983. The English–Wabigoon River system: I. A synthesis of recent research with a view towards mercury amelioration. Can. J. Fish. Aquat. Sci. 40: 2206–2217.

Salonen, K., L. Arvola, T. Tulonen, T. Hammar, T.-R. Metsälä, P. Kankaala, and U. Münster. 1992*a*. Planktonic food chains of a highly humic lake. I. A mesocosm experiment during the spring primary production maximum. Hydrobiologia, 229: 125–142.

Salonen, K., P. Kankaala, T. Tulonen, T. Hammar, M. James, T.-R. Metsälä, P. Kankaala, and L. Arvola. 1992*b*. Planktonic food chains of a highly humic lake. II. A mesocosm experiment in summer during dominance of heterotrophic processes. Hydrobiologia, 229: 143–157.

Sundh, I., and R.T. Bell. 1992. Extracellular dissolved organic carbon released from phytoplankton as a source of carbon for heterotrophic bacteria in lakes of different humic content. Hydrobiologia, 229: 93–106.

Verta, M. 1984. The mercury cycle in lakes; some new hypotheses. Aqua Fenn. 14: 215–221.

Verta, M., T. Matilainen, P. Porvari, M. Niemi, A. Uusi-Rauva, and N.S. Bloom. 1994. Methylmercury sources in boreal lake ecosystems.*In* Mercury pollution: integration and synthesis. *Edited by* C.J. Watras and J.W. Huckabee. Lewis Publishers, Inc., Chelsea, Mich. pp. 119–136.

Westling, O. 1991. Mercury in runoff from drained and undrained peatlands in Sweden. Water Air Soil Pollut. 56: 419–426.

Winfrey, M., and J.W.M. Rudd. 1990. Environmental factors affecting the formation of methylmercury in low pH lakes: a review. Environ. Toxicol. Chem. 9: 853–869.

Xun, L., N.E.R. Campbell, and J.W.M. Rudd. 1987. Measurements of specific rates of net methyl mercury production in the water column and surface sediments of acidified and circumneutral lakes. Can. J. Fish. Aquat. Sci. 44: 750–757.

Population decline of the Japanese sardine *Sardinops melanostictus* owing to recruitment failures

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Abstract: The Japanese sardine *Sardinops melanostictus* started to decline after 1989. Recruitment to age 1 population was small in four year-classes from 1988 to 1991. The population decline after 1989 resulted from recruitment failures in 4 consecutive years. Egg production was high in the years of poor recruitment. The recruitment failures were caused not by a reduction in reproductive output but by low survival between egg stage and age 1 recruitment. Abundance of post first-feeding larvae positively correlated with egg and yolksac larval abundance. Mortality at the first-feeding stage was not so variable as to destroy correlations between the abundance of early life stages. The population of age 1 recruits did not correlate with the abundance of post first-feeding larvae. Recruitment of the sardine was not fixed by the end of the first-feeding stage. Cumulative mortality through the early life stages, rather than relatively instantaneous mortality at the first-feeding stage, is thought to be responsible for the recruitment success or failure and eventual population fluctuations of the sardine.

Résumé : Les effectifs du pilchard du Japon (*Sardinops melanostictus*) ont commencé à baisser après 1989. Le recrutement à la population d'âge 1 a été faible chez quatre classes d'âge, soit celles de 1988 à 1991. Après 1989, la population a diminué parce que le recrutement a été insuffisant pendant 4 années consécutives. La ponte a été importante les années de faible recrutement. L'insuffisance du recrutement n'a pas été causée par une baisse de reproduction, mais bien par la faible survie entre le stade de l'oeuf et l'âge 1 de recrutement. L'abondance des larves ayant dépassé le début de l'alimentation est en corrélation positive avec l'abondance des oeufs et des larves vésiculées. La mortalité au début de l'alimentation ne présentait pas une variabilité suffisante pour supprimer la corrélation entre les valeurs d'abondance des premiers stades du cycle vital. La population de recrues d'un an ne corrélait pas avec l'abondance des larves ayant dépassé le stade du début de l'alimentation. Le recrutement ne s'était pas stabilisé à la fin du stade du début de l'alimentation. On pense que l'importance du recrutement et, par conséquent, les fluctuations des populations de pilchards, dépendent de la mortalité cumulative des premiers stades du cycle vital, non d'une mortalité relative instantanée au stade du début de l'alimentation.

Introduction

Total catch of the Japanese sardine *Sardinops melanostictus* by Japan, Russia, and Korea peaked at 5.43 million metric tonnes (t) in 1988 and started to decline from 1989 to 2.49 million t in 1992 (Food and Agriculture Organization of the United Nations 1994). The decline was most drastic in the Doto region off eastern Hokkaido (Fig. 1), one of the major purse seine fishing grounds of the sardine in the 1980s that constitutes the northernmost part of the grounds on the Pacific side of Japan. The catch in the region peaked at 1.21 million t in 1987 but declined to 1.14 thousand t in 1993 (Mihara et al. 1988, 1994; Fig. 2). The precipitous decline of the catch was accompanied by a drastic change in the age composition of sardines caught in this region (Murakami and Kobayashi 1977, 1978, 1980; Murakami et al. 1980; Kobayashi and Nakata 1981; Nagasawa et al. 1982, 1983, 1984, 1985; Yoshida et al. 1986, 1987; Mihara et al. 1988, 1989, 1990, 1991, 1992, 1993). The average age of the sardines increased greatly during 1988 and 1992 (Fig. 3).

The population of age 1 recruits available for purse seine fishery in the Doto region in the summer and autumn fishing seasons can be used as an index of year-class strength (Wada 1988). Large year-classes appeared in 1980, 1983, and 1986 (Wada 1988; National Research Institute of Fisheries Science 1989), and sustained the large sardine population until 1988. However, year-class strengths after 1988 were low (National Research Institute of Fisheries Science 1993). Recruitment of sardines failed in the 4 consecutive years after 1988 and resulted in a drastic increase in the average age of landed sardines (Fig. 3).

The population of the Japanese sardine in the 1930s reached its first peak in the current century. The annual catch by Japan and Korea totaled about 2 million t between

Received August 18, 1994. Accepted March 2, 1995.
J12546

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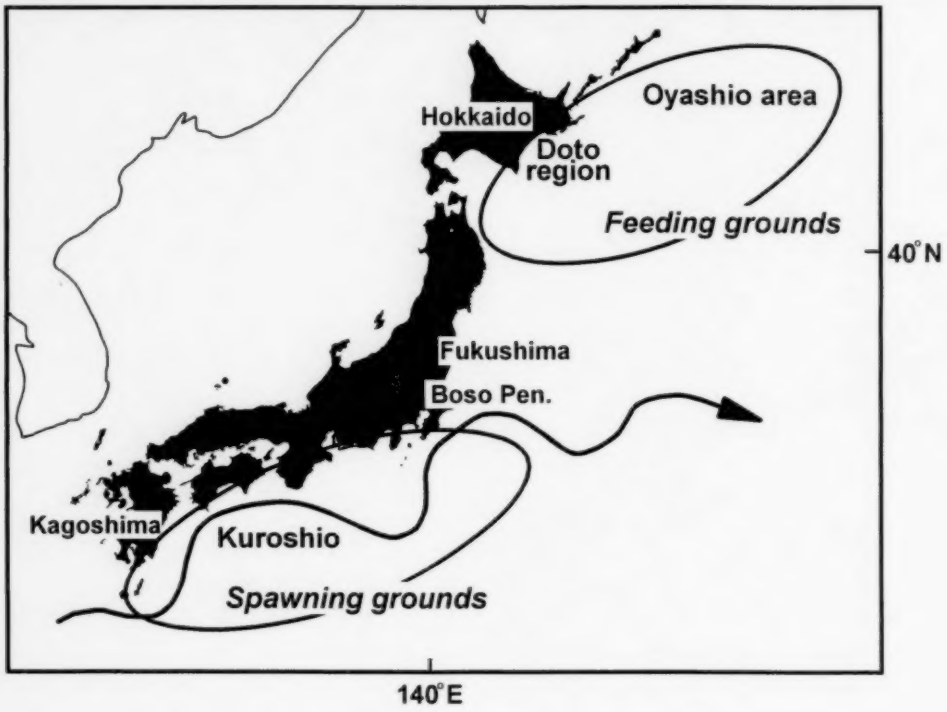
Fig. 1. Spawning grounds along the Kuroshio current and feeding grounds in the Oyashio area of the Japanese sardine on the Pacific coast of Japan. The sardine migrates north in spring for feeding and south in autumn for spawning. The Doto region is located in the feeding grounds and constitutes the northernmost fishing grounds of the sardine in summer and early autumn in years of high population.



1934 and 1939. In the first half of the 1940s, however, it declined rapidly to 0.16 million t in 1945 (Kurita 1957). This population decline was attributed to recruitment failures caused by low survival in the larval stage (Nakai 1962*a*). However, data on egg and larval abundance in the first half of the 1940s were limited and it was difficult to document what had happened to the sardine population in the early 1940s.

The Californian sardine *Sardinops caeruleus* also declined in the 1940s and 1950s. Clark and Marr (1955) found very poor 1949 and 1950 year-classes as a cause of the population reduction. There was much debate on the causes of the recruitment failures and population decline (Schaefer 1954; Clark and Marr 1955; Cushing 1971; Murphy 1977), most of which were discussed from a viewpoint of overfishing.

In principle, two explanations are possible for a recruitment failure. One is low egg production from a reduced spawning biomass and (or) a low spawning activity of adult fish, and the other is high mortality in the early life stages before recruitment to a fishing stock. Kawasaki (1993) reviewed the process of population recovery and decline of the Japanese sardine and stated that the recent decline was caused by survival failure of the larvae. To test this hypothesis and to find out which stage is responsible for survival failures, we estimated the abundance of eggs and larvae and examined correlations in abundance between the stages of egg, yolksac larva, post first-feeding larva, and age 1 recruitment in the years of the sardine population increase and decline on the Pacific side of Japan.

Methods

Egg and larval census

Eggs and larvae of the sardine on the Pacific side of Japan have been censused since 1978 by the two National Fisheries Research Institutes and the 15 Prefectural Fisheries Experimental Stations located on the Pacific coast between Kagoshima and Fukushima. The 15 years' worth of data from the 1978 to 1992 census statistics were analysed in this study.

Vertical tows of a conical net with a side length of 80 cm (Nakai 1962*b*) (1978–1987) or a cylindrical conical net with a side length of 65 cm at the cylindrical part and 130 cm at the conical part (Mori 1992) (1988–1992) were used for ichthyoplankton collection. Both types of net have a 45 cm inside mouth diameter and a mesh aperture of 0.33 mm. A conical net with a 60 cm inside mouth diameter, length 150 cm, and mesh aperture of 0.33 mm (Nakai 1962*b*) was used by the two National Research Institutes during 1978–1990. The nets were equipped with a flowmeter to standardize the filtering efficiency and the length of the tow path of the net (Mori 1992). They were retrieved vertically at 1 m/s from 150 m depth or from the bottom at

Fig. 2. Total catch of the Japanese sardine in the Doto region (after Murakami and Kobayashi 1976, 1977, 1978, 1980; Murakami et al. 1980; Kobayashi and Nakata 1981; Nagasawa et al. 1982, 1983, 1984, 1985; Yoshida et al. 1986, 1987; Mihara et al. 1988, 1989, 1990, 1991, 1992, 1993, 1994).

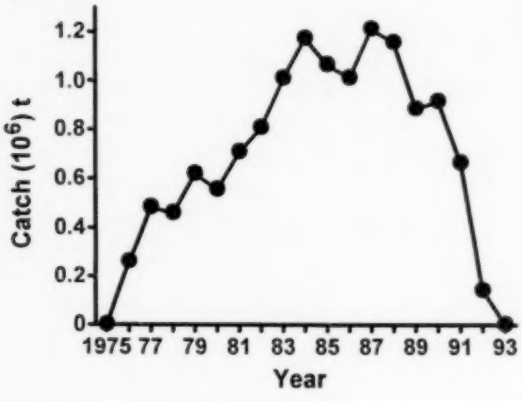
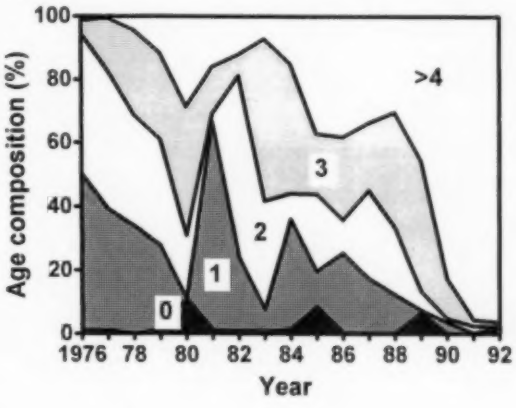


Fig. 3. Age composition of sardines caught in the Doto region (after Murakami and Kobayashi 1977, 1978, 1980; Murakami et al. 1980; Kobayashi and Nakata 1981; Nagasawa et al. 1982, 1983, 1984, 1985; Yoshida et al. 1986, 1987; Mihara et al. 1988, 1989, 1990, 1991, 1992, 1993).



stations shallower than 150 m. Konishi (1980) reported that the majority of sardine eggs and larvae were distributed in a water stratum of 0–100 m depth through night and day. Tow samples were preserved in 5% seawater Formalin. The number of total tow samples in a year ranged from 4262 to 4855 and covered an area from the southwest of Kagoshima to the east of Fukushima on the Pacific side of Japan. This sampling was considered to give a sufficiently precise data set to represent egg and early larval abundance in the spawning grounds. Sardine eggs and larvae were sorted from tow samples. Larvae were classified as yolksac larvae and feeding larvae by examining the presence of yolksac under a dissecting microscope. Total length (TL) of feeding larva was measured to the nearest 0.1 mm. The numbers of eggs, yolksac larvae, and feeding larvae were recorded in 2 mm size intervals for TL, as follows: <6.0 mm, 6.0–7.9 mm, ..., 18.0–19.9 mm, and ≥20 mm. A vertical profile of sea temperature was recorded at every tow station. Sea surface temperature was used for calculating the egg incubation time and yolksac stage duration, as water columns are usually well mixed vertically in the upper 100 m stratum in which sardine eggs and larvae are distributed (Konishi 1980).

Abundance of eggs and larvae

The entire survey area was divided into 30′ latitude by 30′ longitude squares and each year's tow samples were assigned to the square of origin. The densities of eggs, yolksac larvae, and feeding larvae of 6.0–7.9 mm TL in the i-th square in month j, $\bar{x}_{ij}$, $\bar{y}_{ij}$, and $\bar{z}_{ij}$, respectively, were calculated by averaging the densities of eggs or larvae collected by tows in each square, and expressed as the number per square metre of sea surface area. The incubation time standardized egg abundance of month j, E_j, in the entire survey area was calculated as

$$[1] \quad E_j = D_j \cdot \sum_{i=1}^{m} \left(\frac{\bar{x}_{ij}}{I_{ij}} \cdot a_i \right)$$

where D_j is the number of days in month j, and a_i is the area of the i-th square in square metres. I_{ij} is the egg incubation time in days in the i-th square in the month j, which is calculated by Arrhenius' equation after Watanabe (1983) as

$$[2] \quad I_{ij} = 9.86 \cdot \exp\left(\frac{21900}{2} \cdot \frac{1}{\bar{t}_{ij} + 273} \right) \cdot 10^{-17}$$

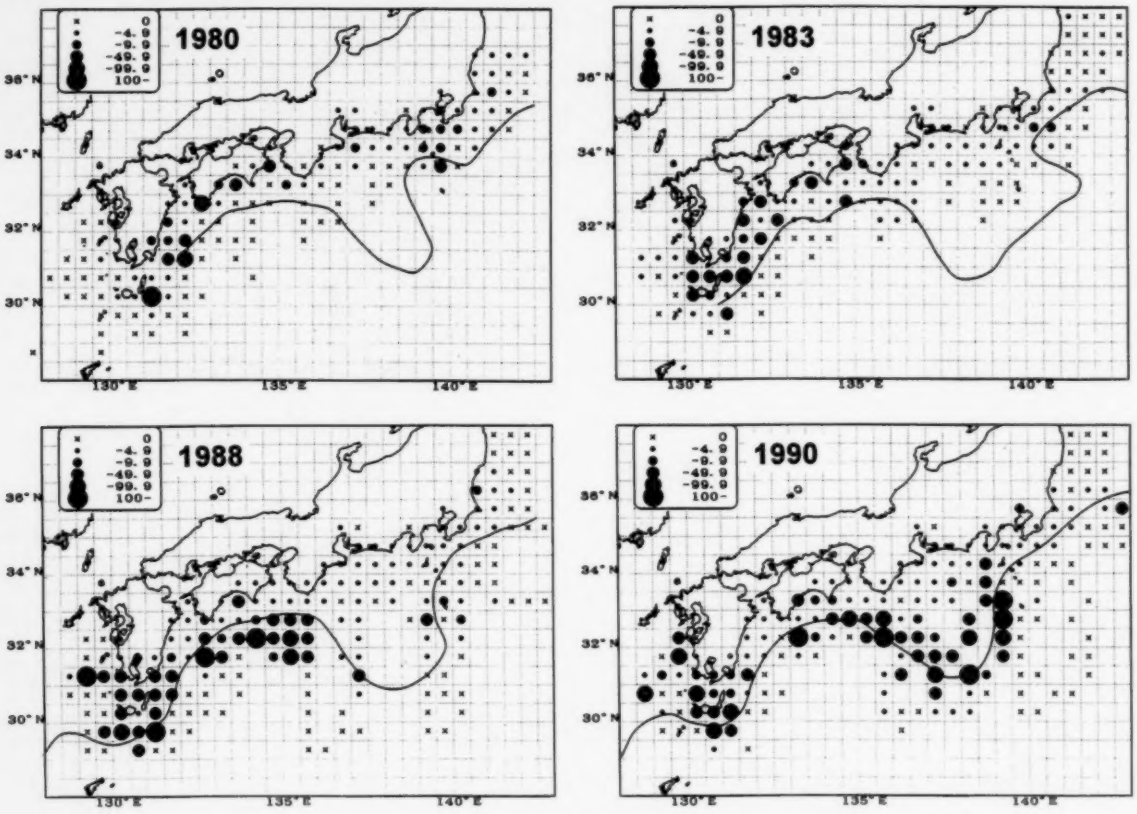
where $\bar{t}_{ij}$ is the average sea surface temperature weighted by egg density at all of the net tow stations in the i-th square in month j. The range of $\bar{t}$ in the main spawning months, February and March, was 12–22°C, which corresponded to 4.8–1.3 days in incubation time I.

The stage duration standardized yolksac larval abundance of month j, L_j, was calculated by the same procedure as was egg abundance. As the duration of the yolksac stage is almost equal to the egg incubation time in *Sardinops* species (Lasker 1964), the duration of the yolksac stage in days under the average sea surface temperature weighted by yolksac larval density at all of the net tow stations in the i-th square in month j, Y_{ij}, was calculated by eq. 2. L_j was calculated as

$$[3] \quad L_j = D_j \cdot \sum_{i=1}^{m} \left(\frac{\bar{y}_{ij}}{Y_{ij}} \cdot a_i \right)$$

In this study we used the abundance of feeding larvae of 6.0–7.9 mm TL as a population index of post first-feeding larvae. As the Formalin-preserved TL of laboratory-reared first-feeding sardines is 5.7 mm, and Formalin preservation causes TL to shrink by 5% (Matsuoka and Mitani 1989), the live TL of the first-feeding sardines was estimated to be 6.0 mm. Theilacker (1980) demonstrated a 20% shrinkage in body length of 5-mm northern anchovy (*Engraulis mordax*) larvae caused by net abrasion and Formalin preservation; we therefore estimated that the TL of a field-caught first-feeding sardine would be 4.8 mm after Formalin preservation. We do not have data on larval

Fig. 4. Distribution of sardine eggs on the Pacific side of Japan. The location of the Kuroshio current axis in the main spawning month (the second half of February for 1980, 1983, and 1990, and the first half of March for 1988) is indicated by the solid line (after Maritime Safety Agency 1980, 1983, 1988, 1990). Solid circles and crosses show the annual egg abundance in 30′ × 30′ squares (in trillions).



growth rate in the years studied. The average growth rate of sardine larvae of 6–20 mm in the Kuroshio area was reported to be 0.35–0.95 mm (field-caught and ethanol-preserved TL)·day^{-1} (Watanabe et al. 1991). Larvae with a preserved TL of 6.0–7.9 mm are estimated to be 2.4 to 6.4 days after the initiation of feeding, using the growth rate of 0.5 mm·day^{-1} in the early feeding stage. The duration of the 6.0–7.9 mm size-class was determined to be 4 days using the growth rate of 0.5 mm·day^{-1}. The size-class duration standardized abundance of post first-feeding larvae in month j, F_j, was calculated as

$$[4] \quad F_j = \frac{D_j}{4} \cdot \sum_{i=1}^{m} (\bar{z}_{ij} \cdot a_i)$$

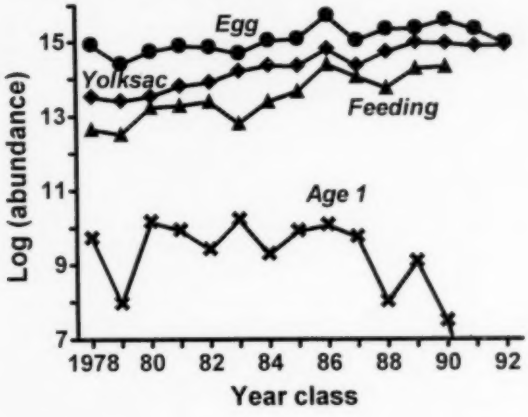
The annual abundance of eggs, yolksac larvae, and post first-feeding larvae was calculated by summing the monthly abundance from October to the following September, because sardine spawning starts in November and ceases in July or August with a peak in February and March (Mori et al. 1988). The coefficients of variance of annual egg abundance were 4.5–32.7%, and those of annual larval abundance were smaller.

Results

The spawning grounds of the sardine extended along the Kuroshio current from the south of Kagoshima to the east of Boso Peninsula on the Pacific side of Japan (Fig. 4). The distribution of sardine eggs was limited to the inshore area of the Kuroshio current in 1980 and 1983. The distribution expanded offshore in 1988. Offshore expansion and westerly shift of the spawning grounds were characteristic features in the second half of the 1980s (Kawai 1994). Sardine eggs were concentrated in the area along the meandering Kuroshio current in 1990.

The egg abundance standardized for incubation time in 1979 was 250 trillion. It gradually increased through the first half of the 1980s and markedly increased to 5130 trillion in 1986. Abundance remained high in the second half of the 1980s and in 1991 and 1992 (Fig. 5). The annual average egg abundance during the 4 years of recruitment failure, 1988–1991, was 2610 ± 690 trillion, which was much higher than the 800 ± 230 trillion in the 5 years of recruitment success, 1980, 1981, 1983, 1985, and 1987. Recruitment failures in 1988–1991 were not caused by a reduction in egg production.

Fig. 5. Abundance of eggs (egg), yolksac larvae (yolksac), and post first-feeding larvae (feeding) standardized for stage duration time, and available population of age 1 recruits (age 1) in the Doto region. (Wada 1988; National Research Institute of Fisheries Science 1989, 1993). The abundance of post first-feeding larvae in 1991 and 1992 was not available because the TL of feeding larvae was not measured in these years. The available population of age 1 recruits in 1991 was estimated to be 0 and that in 1992 could not be estimated because the total catch in the Doto region was too small, 1.1 thousand t, in 1993 (Mihara et al. 1994).



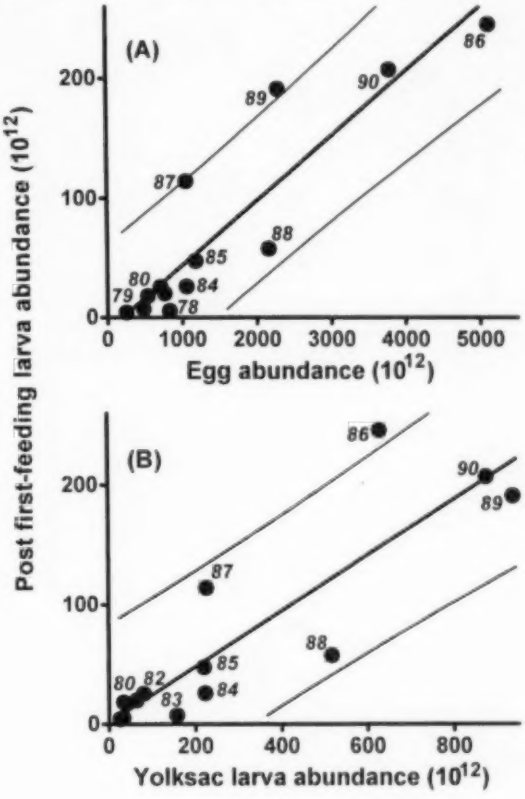
The abundance of yolksac and post first-feeding larvae standardized for stage duration time increased with the increase in egg abundance through the 1980s (Fig. 5). Yolksac larval abundance was 25 trillion in 1979 and increased to 500–900 trillion in 1988–1992. The abundance of post first-feeding larvae increased from 3 trillion in 1979 to 210 trillion in 1990. Available populations of age 1 recruits were 14, 16, and 12 billion in 1980, 1983, and 1986, respectively. These were the three largest year-classes in the 1980s (Wada 1988; National Research Institute of Fisheries Science 1989; Fig. 5). The population of the 1988 year-class was estimated to be 0.1 billion and that of the 1991 year-class was almost zero. The percentage of age 1 fish (1991 year-class) was zero in the 140 thousand t of sardines caught in the Doto region in 1992 (Mihara et al. 1993). Although the abundance of sardines in the three early life stages was high, the available population of age 1 recruits decreased after 1988 (Fig. 5).

The abundance of post first-feeding larvae positively correlated with egg abundance (Fig. 6A). A positive correlation was also detected between the abundance of the post first-feeding larvae and yolksac larvae (Fig. 6B). The available population of age 1 recruits, however, did not correlate with the abundance of post first-feeding larvae (Fig. 7).

Discussion

In the 1940s and 1950s, both the Japanese and Californian sardine populations collapsed, resulting in much argument as to the causes. A common feature of the two populations was that recruitment failed in successive years, as

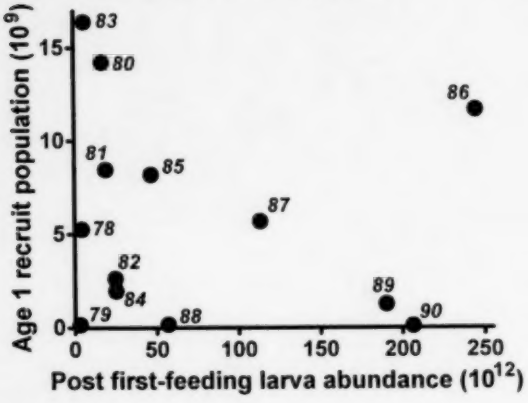
Fig. 6. Regression (thick line) of the abundance of post first-feeding larvae on eggs (A) and yolksac larvae (B), with 95% confidence limits (thin lines). (A) $Y = -10.4 + 0.0539X$, $r^2 = 0.830$, $n = 13$, $P < 0.001$. (B) $Y = 1.50 + 0.233X$, $r^2 = 0.774$, $n = 13$, $P < 0.001$.



in the recent decline of the Japanese sardine population. The question was whether these recruitment failures were caused by density-independent environmental changes or a process dependent on the adult population density. Kurita (1957), Nakai (1962*a*), and Marr (1963) thought that environmental changes caused the failures. Schaefer (1954), Cushing (1971), Murphy (1977), and Ricker (1977) found a density-dependent recruitment process and assumed that the population collapse was caused by overfishing. Cushing (1988) stated that since the 1950s many pelagic fish stocks have suffered from recruitment overfishing, which results in a reduction of recruitment with increasing fishing intensity (Cushing 1977).

The number of government-licenced large- and medium-sized purse seiners operating in the Doto region has been limited to 24. The number of purse seiners at work had been 24 until 1991, and then decreased to 22 in 1992 because of the decline of the sardine stock (Nagasawa et al. 1983; Mihara et al. 1993). Fishing intensity had been almost stable in the years of the population increase and decrease. If growth overfishing, which results in a decrease in mean fish size with increasing fishing intensity (Gulland

Fig. 7. Correlation of available population of age 1 recruits in the Doto region (after Wada 1988; National Research Institute of Fisheries Science 1989, 1993) with the abundance of post first-feeding larvae.



1982), had caused the recent population decline of the Japanese sardine under nearly constant fishing intensity, older age groups of large size would have disappeared first from the population and a reduction in the average size and age of the population would have resulted. However, most of the sardines landed after 1988 were of the 1987 and earlier year-classes (Fig. 3). Even the catch in 1991 remained mostly from the 1987 and earlier year-classes of large size, accounting for 95.6% of the total (Mihara et al. 1992). If the recruitment overfishing (Cushing 1977) had induced the population reduction, egg abundance in the years of 1988–1991 would have been much lower than in the years of recruitment success before 1987, but egg abundance remained high, and was even higher than in the years of recruitment success during the early and mid-1980s. Clearly the recruitment failures were not induced by a decrease in reproductive output from adult fish. We conclude that neither growth overfishing nor recruitment overfishing was responsible for the recent recruitment failures and population decline of the Japanese sardine. The decline of the sardine population in the northwestern Pacific was brought about by extremely low survival after enormous egg production.

Soutar and Isaacs (1969) first reported scale aggregations of the Pacific sardine *S. caerulea* in a piston core of sea floor sediments in the anaerobic Santa Barbara basin in the Southern California Bight. They found 12 occurrences of the sardine over the past 1850 years. Baumgartner et al. (1992) reconstructed the history of biomass fluctuations of the sardine and northern anchovy *Engraulis mordax* since 270 AD on the basis of the deposition rate of scales onto the bottom of the Santa Barbara Basin. They demonstrated that the sardine and anchovy populations repeatedly collapsed and recovered over the preceding 1700 years. These studies show that population fluctuations are a natural phenomenon that occur independently of human fishing.

Lasker (1985) mentioned that the solution to the stock-recruitment problem is to determine the minimum population for a species that ensures its recovery. However, he also stated that there was no definable minimum stock size common to clupeoid recoveries. A large recruitment can result from a small adult population, as in the Japanese sardine, which resurged from a very small population (Kondo 1980) to one capable of sustaining a 5 million t fishery. Recruitment failure can occur after high production of eggs as in the cases of the Japanese sardine in 1988–1991 in this study, and of the 1975 year-class of the northern anchovy (Lasker 1981; Lo 1985). Koslow (1992) simulated the stock-recruitment relationship and found that the relationship cannot be defined in species of high fecundity such as marine fishes because small variations in natural mortality lead to large changes in adult population.

Sardine eggs hatch in 2–3 days and newly hatched larvae start feeding after exhausting their yolk in another 2–3 days (Matsuoka and Mitani 1989). The first-feeding stage has been recognized as the critical period for larval survival and eventual recruitment of a new cohort (Hjort 1914). The abundance of post first-feeding larvae positively correlated with both the egg and yolksac larval abundance (Fig. 6). This indicates that mortality at the first-feeding stage was not so variable as to destroy the positive correlation of abundance between the stages before and after this stage. The recruitment failures of the 4 consecutive years could not be attributed to a low survival at the first-feeding stage.

As the mouth diameter of the net used in this study was 45 or 60 cm with bridles in front of the mouth, some feeding larvae may have avoided the net, especially in the daytime (Smith and Richardson 1977). More than 90% of the feeding larvae collected by the nets were smaller than 10 mm. The annual average TL of the feeding larvae collected ranged from 6.1 mm in 1983 to 7.1 mm in 1987. As we did not correct catch data for a possible night–day difference in catch efficiency, the abundance of post first-feeding larvae might have been underestimated; percentages of daytime tows in annual total tows were almost the same between years under the formulated scheme of net sampling in this study.

We might need to consider the possibility that the relatively high correlations between the abundance of eggs and yolksac larvae with that of post first-feeding larvae are attributable to three abundance estimates that originated from the same net sampling. The Kuroshio current goes through the sardine spawning grounds faster than 2 knots in its axial path (Taft 1972), and transports pelagic eggs and larvae eastward as fast as 100 km·day^{-1}. Distribution patterns of sardine eggs, yolksac larvae, and feeding larvae were different within a spawning ground (Kuroda 1991). The three abundance estimates were considered to be relatively independent of each other in such a strong advection field. The coverage of the net sampling stations over the sardine spawning in time and space in this study is large enough to represent sardine populations in early life stages.

The available population of the age 1 recruits in the Doto region, which represents a year-class strength and can be an index of recruitment (Wada 1988), did not correlate with the post first-feeding larval abundance (Fig. 7). Recruitment was not fixed by the end of the first-feeding stage. Mortality after the first-feeding stage determined

the population of age 1 recruits of the sardine. Peterman et al. (1988) found that mortality of first-feeding larvae was not a strong determinant of age 1 recruit abundance in the northern anchovy. Butler (1991) showed that sardine recruitment at age 2 did not correlate with larval mortality rates. Kuroda (1991) suggested that survival variability in white bait (late larva of 15–35 mm TL) and juvenile stages might have determined age 1 population in the Japanese sardine. Cumulative mortality between the egg stage and age 1 recruitment seemed to determine the year-class strength rather than the relatively instantaneous mortality at Hjort's (1914) critical period. Houde (1989) mentioned that while high fecundities of marine fishes permit large losses of eggs and larvae by episodic events with relatively minor implications for recruitment, subtle changes in mean vital rates through the early life period for 50–70 days can have major consequences.

The Kuroshio current has strong effects on the spawning environments of the sardine. Nakata et al. (1994) found that chlorophyll *a* concentrations and densities of small copepods were low in the years when the Kuroshio had a stable meandering path such as 1977–1980 and 1988 compared with those in which the Kuroshio did not meander. Among the years of low production of phyto- and zooplankton, a dominant year-class was established in 1980 and recruitment failed in 1979 and 1988. This might indicate that recruitment of the sardine was not determined only by survivorship in the spawning grounds. We must focus our attention on later life stages in the downstream waters of the Kuroshio east of the Boso Peninsula if we are to find the recruitment-determining processes of the sardine.

Acknowledgments

We thank T. Kawasaki, K. Mori, T. Kishida, and Y. Oozeki for their helpful comments and critiques on the manuscript. Our thanks are extended also to M. Ishida and those who have been involved in the egg and larval censuses taken on the Pacific side of Japan. This study was supported in part by a Grant-in-Aid (Bio-Cosmos Program) from the Ministry of Agriculture, Forestry, and Fisheries (BCP-95-IV-A-5). This work is contribution A No. B-108 from the National Research Institute of Fisheries Science.

References

Baumgartner, T.R., A. Souter, and V. Ferreira-Bartrina. 1992. Reconstruction of the history of Pacific sardine and northern anchovy populations over the past two millennia from sediments of the Santa Barbara Basin, California. Calif. Coop. Oceanic Fish. Invest. Rep. 33: 24–40.

Butler, J.L. 1991. Mortality and recruitment of Pacific sardine, *Sardinops sagax caerulea*, larvae in the California current. Can. J. Fish. Aquat. Sci. 48: 1713–1723.

Clark, F.N., and J.C. Marr. 1955. Population dynamics of the Pacific sardine. *In* Calif. Coop. Oceanic Fish. Invest. Prog. Rep., 1 July 1953 to 31 March 1955. pp. 13–48.

Cushing, D.H. 1971. The dependence of recruitment on parent stock in different groups of fishes. J. Cons. Cons. Int. Explor. Mer 33: 340–362.

Cushing, D.H. 1977. The problems of stock and recruitment. *In* Fish population dynamics. *Edited by* J.A. Gulland. John Wiley & Sons, Chichester. pp. 116–133.

Cushing, D.H. 1988. The study of stock and recruitment. *In* Fish population dynamics. 2nd. ed. *Edited by* J.A. Gulland. John Wiley & Sons, Chichester. pp. 105–128.

Food and Agriculture Organization of the United Nations. 1994. Yearbook of fishery statistics. catches and landings. Vol. 74. Food and Agriculture Organization of the United Nations, Rome, Italy.

Gulland, J.A. 1982. Fish stock assessment. John Wiley & Sons, Chichester.

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, 20: 1–228.

Houde, E.D. 1989. Subtleties and episodes in the early life of fishes. J. Fish Biol. 35(Suppl. A): 29–38.

Kawai, T. 1994. Analysis of the relationship between the replacement of fish species in 1988 and changes of spawning period or spawning ground of the Japanese sardine, *Sardinops melanostictus*. Bull. Tohoku Natl. Fish. Res. Inst. 56: 77–90.

Kawasaki, T. 1993. Recovery and collapse of the Far Eastern sardine. Fish. Oceanogr. 2: 244–253.

Kobayashi, T., and J. Nakata. 1981. Stock assessment of coastal resources. Annual report for fiscal 1980, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 48–53.

Kondo, K. 1980. The recovery of the Japanese sardine: the biological basis of stock-size fluctuations. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, 177: 332–354.

Konishi, Y. 1980. Vertical distribution of eggs and larvae of sardine, *Sardinops melanosticta* (T. et S.), and round herring, *Etrumeus micropus* (T. et S.). Bull. Nansei Reg. Fish. Res. Lab. 12: 93–103.

Koslow, J.A. 1992. Fecundity and the stock-recruitment relationship. Can. J. Fish. Aquat. Sci. 49: 210–217.

Kurita, S. 1957. Causes affecting the size of the sardine stock in the waters of Japan and adjacent regions: with particular reference to the catch declining since 1941. Bull. Tokai Reg. Fish. Res. Lab. 18: 1–14.

Kuroda, K. 1991. Studies on the recruitment process focusing on the early life history of the Japanese sardine, *Sardinops melanostictus* (Schlegel). Bull. Natl. Res. Inst. Fish. Sci. (Jpn.), 3: 25–278.

Lasker, R. 1964. An experimental study of the effect of temperature on the incubation time, development, and growth of Pacific sardine embryos and larvae. Copeia, 1964: 399–405.

Lasker, R. 1981. Factors contributing to variable recruitment of the northern anchovy (*Engraulis mordax*) in the California Current: contrasting years, 1975 through 1978. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, 178: 375–388.

Lasker, R. 1985. What limits clupeoid production? Can. J. Fish. Aquat. Sci. 42(Suppl.): 31–38.

Lo, N.C.H. 1985. Egg production of the central stock of northern anchovy. Fish. Bull. 83: 137–150.

Maritime Safety Agency. 1980. Current profile. *In* Quick bulletin of ocean conditions. No. 5. Maritime Safety Agency, Tokyo.

Maritime Safety Agency. 1983. Current profile. *In* Quick bulletin of ocean conditions. No. 5. Maritime Safety Agency, Tokyo.

Maritime Safety Agency. 1988. Current profile. *In* Quick bulletin of ocean conditions. No. 6. Maritime Safety Agency, Tokyo.

Maritime Safety Agency. 1990. Current profile. *In* Quick bulletin of ocean conditions. No. 5. Maritime Safety Agency, Tokyo.

Marr, J.C. 1963. A model for the population biology of the Pacific sardine *Sardinops caerulea*. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, 154: 270–278.

Matsuoka, M., and T. Mitani 1989. Hatching and rearing experiments on eggs of Japanese sardine, *Sardinops melanostictus*, collected from adjoining area of the Nagasaki harbor (preliminary study). Bull. Seikai Reg. Fish. Res. Lab. 67: 15–22.

Mihara, Y., J. Nakata, Y. Imai, and M. Sano. 1988. Stock assessment of coastal resources. Annual report for fiscal 1987, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 26–36.

Mihara, Y., Y. Imai, H. Taka, and K. Yoda. 1989. Stock assessment of sardine and mackerel. Annual report for fiscal 1988, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 79–89.

Mihara, Y., H. Taka, Y. Imai, T. Honma, and K. Yoda. 1990. Stock assessment of sardine and mackerel. Annual report for fiscal 1989, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 68–78.

Mihara, Y., H. Taka, Y. Imai, T. Honma, and K. Yoda. 1991. Sardine and mackerel. Annual report for fiscal 1990, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 87–98.

Mihara, Y., H. Taka, T. Honma, and K. Yoda. 1992. Sardine and mackerel. Annual report for fiscal 1991, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 66–77.

Mihara, Y., H. Taka, T. Honma, and T. Yamagishi. 1993. Sardine and mackerel. Annual report for fiscal 1992, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 52–65.

Mihara, Y., H. Taka, T. Honma, and T. Yamagishi. 1994. Sardine and mackerel. Annual report for fiscal 1993, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 50–61.

Mori, K. 1992. Vertical tow net with a small mouth opening. *In* Manual for pelagic fish egg and larval survey. *Edited by* S. Kume. National Research Institute of Fisheries Science, Tokyo. pp. 8–14.

Mori, K., K. Kuroda, and Y. Konishi. 1988. Monthly egg productions of the Japanese sardine, anchovy, and mackerels off the southern coast of Japan by egg censuses: January, 1978 through December, 1986. Datum Collect. Tokai Reg. Fish. Res. Lab. 12: 1–321.

Murakami, K., and T. Kobayashi. 1976. Stock assessment of coastal resources. Annual report for fiscal 1975, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 62–68.

Murakami, K., and T. Kobayashi. 1977. Stock assessment of coastal resources. Annual report for fiscal 1976, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 55–62.

Murakami, K., and T. Kobayashi. 1978. Stock assessment of coastal resources. Annual report for fiscal 1977, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 83–92.

Murakami, K., and T. Kobayashi. 1980. Stock assessment of coastal resources. Annual report for fiscal 1978, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 127–134.

Murakami, K., T. Kobayashi, and J. Nakata. 1980. Stock assessment of coastal resources. Annual report for fiscal 1979, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 108–114.

Murphy, G.I. 1977. Clupeoids. *In* Fish population dynamics. *Edited by* J.A. Gulland. John Wiley & Sons, Chichester. pp. 116–133.

Nagasawa, K., J. Nakata, and T. Kobayashi. 1982. Stock assessment of coastal resources. Annual report for fiscal 1981, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 42–53.

Nagasawa, K., J. Nakata, and T. Kobayashi. 1983. Stock assessment of coastal resources. Annual report for fiscal 1982, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 57–69.

Nagasawa, K., J. Nakata, Y. Imai, and H. Kondo. 1984. Stock assessment of coastal resources. Annual report for fiscal 1983, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 31–44.

Nagasawa, K., J. Nakata, Y. Imai, and H. Kondo. 1985. Stock assessment of coastal resources. Annual report for fiscal 1984, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 39–48.

Nakai, Z. 1962*a*. Studies relevant to mechanisms underlying the fluctuation in the catch of the Japanese sardine, *Sardinops melanosticta* (Temminck & Schlegel). Jpn. J. Ichthyol. 9: 1–115.

Nakai, Z. 1962*b*. Apparatus for collecting macroplankton in the spawning surveys of *iwashi* (sardine, anchovy, and round herring) and others. Bull. Tokai Reg. Fish. Res. Lab. 9: 221–237.

Nakata, K., A. Hada, and Y. Matsukawa. 1994. Variations in food abundance for Japanese sardine larvae related to the Kuroshio meander. Fish. Oceanogr. 3: 39–49.

National Research Institute of Fisheries Science. 1989. Matures and immatures of sardine Pacific subpopulations. Long-term forecasting on the distribution and abundance of the important fishery resources and the related oceanographic conditions in the sea of Tohoku & Chuo Blocs. 79: 18–22.

National Research Institute of Fisheries Science. 1993. Long-term forecasting on the distribution and abundance of the important fishery resources and the related oceanographic conditions in the sea of Chuo Bloc. 91: 18–22.

Peterman, R., M.J. Bradford, N.C.H. Lo, and R.D. Methot. 1988. Contribution of early life stages to interannual variability in recruitment of northern anchovy (*Engraulis mordax*). Can. J. Fish. Aquat. Sci. 45: 8–16.

Ricker, W.E. 1977. The historical development. *In* Fish population dynamics. *Edited by* J.A. Gulland. John Wiley & Sons, Chichester. pp. 1–26.

Schaefer, M.B. 1954. Some aspects of the dynamics of populations important to the management of the commercial marine fisheries. Bull. Inter-Am. Trop. Tuna Comm. 1: 1–56.

Smith, P.E., and S.L. Richardson. 1977. Standard techniques for pelagic fish egg and larva surveys. FAO Fish. Tech. Pap. 175: 1–100.

Soutar, A., and J.D. Isaacs 1969. History of fish populations inferred from fish scales in anaerobic sediments off California. CalCOFI Rep. 13: 63–70.

Taft, B. 1972. Characteristics of the flow of the Kuroshio south of Japan. *In* Kuroshio. *Edited by* H. Stommel and K. Yoshida. University of Tokyo Press, Tokyo. pp. 165–216.

Theilacker, G.H. 1980. Changes in body measurements of larval northern anchovy, *Engraulis mordax*, and other fishes due to handling and preservation. Fish. Bull. 78: 685–692.

Wada, T. 1988. Population dynamics on Japanese sardine, *Sardinops melanostictus*, caught by the domestic purse seine fishery in the waters off the coast of southeastern Hokkaido. Bull. Hokkaido Reg. Fish. Res. Lab. 52: 1–138.

Watanabe, T. 1983. Spawning survey method. *In* Population dynamics of fishery resources. *Edited by* T. Ishii. Koseisha-Koseikaku, Tokyo. pp. 9–29.

Watanabe, Y., K. Yokouchi, Y. Oozeki, and H. Kikuchi. 1991. Preliminary report on larval growth of the Japanese sardine spawned in the dominant current area. Rep. No. C.M.-ICES 1991/L:33, International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Yoshida, H., J. Nakata, Y. Imai, M. Sano, and Y. Yamashita. 1986. Stock assessment of coastal resources. Annual report for fiscal 1985, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 35–43.

Yoshida, H., J. Nakata, Y. Imai, M. Sano, and Y. Yamashita. 1987. Stock assessment of coastal resources. Annual report for fiscal 1986, Hokkaido Kushiro Fisheries Experimental Station, Japan. pp. 32–40.

Mitochondrial DNA differentiation of sympatric populations of brown trout, *Salmo trutta* L., from Lough Melvin, Ireland

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Abstract: RFLP analysis of mitochondrial DNA (mtDNA) was used to study variation within and between ferox, sonaghen, and gillaroo, the local names of three types of brown trout, *Salmo trutta* L., which live sympatrically in Lough Melvin, northwest Ireland. They spawn in separate rivers or sections of rivers and thus maintain reproductive integrity by their innate homing behaviour. In this study, 12 restriction enzymes were used to survey 4.9% of the genome in 37 trout. Five of the enzymes were informative (*Ava*II, *Hae*III, *Hin*fI, *Mbo*I, and *Xba*I) and these were used to examine an additional 139 trout. Seven mtDNA haplotypes were found in the first 37 trout and one further haplotype, owing to variation at *Mbo*I sites, was found in the larger sample of trout. All of the ferox were monomorphic at all sites examined whereas six haplotypes were found among the gillaroo, three of which were unique to this group. Five haplotypes were found among the sonaghen, two of which were found only in this group. Sonaghen and gillaroo share three haplotypes but they are present in each at different frequencies. Relative to morphological, allozyme, and multilocus probe results, mtDNA provides the most discriminating marker system for these trout.

Résumé : Dans le Lough Melvin (nord-Ouest de l'Irlande), trois types de truite brune (*Salmo trutta* L.), appelés localement ferox, sonaghen et gillaroo, vivent en sympatrie; nous avons étudié certains des RFLP de leur ADN mitochondrial pour caractériser les variations chez un même type et entre les types. Les trois types de truites fraient dans des rivières différentes, ou des parties de rivière différentes, de sorte que, grâce au comportement inné de retour aux eaux natales, leur intégrité reproductive est maintenue. Nous avons utilisé 12 enzymes de restriction pour étudier 4,9% du génome de 37 truites. Cinq de ces enzymes se sont révélées informatives (*Ava*II, *Hae*III, *Hin*fI, *Mbo*I et *Xba*I) : elles ont servi à l'étude de 139 truites additionnelles. Nous avons relevé sept haplotypes d'ADN mitochondrial dans le premier échantillon (37 truites); dans l'autre échantillon, nous avons trouvé un haplotype additionnel, caractérisé par une variation des sites *Mbo*I. Chez tous les sujets du type ferox, les sites étudiés étaient monomorphes; chez le type gillaroo, nous avons trouvé six haplotypes, dont trois particuliers à ce groupe. Enfin, nous avons relevé cinq haplotypes chez les sonaghen, dont deux qui leur sont particuliers. Trois haplotypes se retrouvent tant chez les sonaghen que chez les gillaroo, mais à des fréquences différentes. Par comparaison aux traits morphologiques, aux allozymes et aux caractères révélés par les sondes multilocus, l'ADN mitochondrial est le système marqueur qui permet le mieux de différencier ces truites.
[Traduit par la Rédaction]

Introduction

Three types of brown trout (*Salmo trutta* L.) coexist in Lough Melvin, northwest Ireland. These are known locally as gillaroo, sonaghen, and ferox. They are distinct morphologically (Cawdery and Ferguson 1988) and have different feeding preferences (Ferguson 1986). Allozyme studies have indicated that the three types are reproductively isolated. Thus, only gillaroo possess the *LDH-1*QO* allele, while ferox show a high frequency (0.65) of the *LDH-5*100* allele, which does not exceed a frequency of 0.02 in the other two types. Sonaghen are characterized by relatively high frequencies of the *CK-2*115* and *GPI-2*135* alleles (Ferguson and Mason 1981). This reproductive integrity is maintained by their innate homing abilities. Gillaroo spawn in the only outflowing river of the lake, whereas sonaghen spawn in the inflowing rivers. Ferox spawn in the lower deeper section of one of the inflowing rivers, which is also used further upstream by sonaghen. In view of these differences, it has been proposed that the three types be designated as separate subspecies (Ferguson and Taggart 1991).

Received August 11, 1994. Accepted March 7, 1995.
J12531

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A multilocus DNA fingerprint analysis was carried out on ferox, sonaghen, and gillaroo, using the 33.6 human minisatellite probe (Prodöhl et al. 1992). Although no unambiguous diagnostic fragments were observed for any of the types, lower band sharing coefficients were found in intertype comparisons than in the relevant intratype comparisons. Screening with five single locus minisatellite (VNTR) DNA probes showed that there were from three to eight alleles at each locus in the three types (Prodöhl 1993; Prodöhl et al. 1992). Significant differences were found in the frequencies of these alleles at all loci. However, apart from rare alleles (frequency ≤0.09), no unique alleles were found in any of the three types.

Studies of mitochondrial DNA (mtDNA) variation have proved valuable in studying population genetics and phylogeographic origins in many fish species (Billington and Hebert 1988; Bernatchez and Dodson 1991). Brown trout mtDNA variation has been examined using both sequence and restriction fragment length polymorphism (RFLP) analysis. Direct sequence analysis failed to find any variation in the mitochondrial control region (Bernatchez et al. 1992) or cytochrome *b* and ATPase subunit 6 genes (Giuffra et al. 1994) among brown trout individuals from different parts of the Atlantic basin. A few small-scale RFLP studies have been reported (Palva 1986; Gyllensten and Wilson 1987; McVeigh and Ferguson 1988; Hynes et al. 1989; Ovenden et al. 1993). In all of these studies only a few enzymes and individuals have been examined. For example, McVeigh and Ferguson (1988) reported four mtDNA haplotypes in 15 Melvin brown trout using six restriction enzymes. They suggested that there may be mtDNA haplotypes that are unique to the different types of Melvin trout. This paper reports the results of an extended mtDNA RFLP analysis using a larger number of restriction enzymes and larger sample sizes.

Materials and methods

Trout were obtained from Lough Melvin both by gill-netting adults in the lake and by electro-fishing of juveniles from the appropriate streams in 1986 and 1990. In total 46 ferox, 56 gillaroo, and 74 sonaghen were available for analysis. All lake samples were adults; therefore, it is most unlikely that they were siblings. Further, the juveniles were sampled over large stretches of the streams to minimize the chances of sampling siblings. The whole fish or individual tissues were immediately frozen on dry ice and subsequently stored at −40 or −70°C until required. Lake-caught fish were identified using morphological characters (Ferguson and Taggart 1991). Juvenile gillaroo were obtained from the outflowing river where only this type is known to spawn. Juvenile sonaghen were taken from the inflowing rivers where they spawn apart from the other types. In the case of juvenile ferox, trout were obtained from the lower reaches of the inflowing river where they spawn. This river is also used further upstream by sonaghen; therefore, only those trout homozygous for the diagnostic eye-specific (*LDH-5*100*) allele were used.

Two methods of mtDNA extraction and RFLP detection were used. Initially, mtDNA analysis was carried out as described by McVeigh (1989). This involved the isolation of purified mtDNA by caesium chloride gradient centrifugation. The purified mtDNA was cleaved with restriction enzymes according to the manufacturer's instructions. The resulting fragments were subsequently end-labelled using [α-^{32}P]dNTP and detected by autoradiography. Twenty-five restriction enzymes were used in this phase of the study, namely *Ava*II, *Bam*HI, *Bgl*I, *Bgl*II, *Bsc*I, *Bst*EII, *Dde*I, *Dra*I, *Fnu*DII, *Hae*III, *Hha*I, *Hinc*II, *Hind*III, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, *Mbo*I, *Msp*I, *Pst*I, *Pvu*II, *Rsa*I, *Sma*I, *Taq*I, and *Xba*I.

Twelve fish (4 of each type) were examined with all 25 restriction enzymes. *Bst*EII and *Msp*I yielded fragments that were not easily resolved and thus were not used further. *Fnu*DII and *Hpa*II proved prohibitively expensive and therefore were not available for extensive analysis. On the basis of preliminary results from the first 12 fish examined and on evidence from other studies of salmonid populations (Gyllensten and Wilson 1987) 12 enzymes were used to examine 37 trout (9 ferox, 9 gillaroo, and 19 sonaghen). These were *Ava*II, *Bgl*I, *Bgl*II, *Hae*III, *Hha*I, *Hinc*II, *Hind*III, *Hin*fI, *Mbo*I, *Rsa*I, *Taq*I, and *Xba*I.

The remaining 139 trout were analyzed using total DNA extracted as described by Taggart et al. (1992). Following restriction and electrophoresis, Southern blots of total genomic DNA were probed with α-^{32}P random primed total purified brown trout mtDNA. On the basis of the initial results, only *Ava*II, *Hae*III, *Hin*fI, *Mbo*I, and *Xba*I were used to screen these fish. Patterns for each enzyme were assigned uppercase letters in order of discovery, resulting in a five-letter composite haplotype for each fish. (Additional haplotypes were found in other brown trout samples, hence consecutive numbers are not used here.)

Fragment sizes were determined by comparison with DNA markers. Nucleon diversity within populations was calculated according to Nei and Tajima (1981). The nucleotide sequence divergence between mtDNA haplotypes was estimated using the fragment approach of Nei and Li (1979). The statistical significance of differences in haplotype frequency among the three types and among year-classes was tested using the analysis of molecular variance (AMOVA) test (Excoffier et al. 1992). The degree of genetic differentiation among the three types was analyzed using Wright's statistic θ (equivalent to F_{ST}), using the HAPLOID program as described by Weir (1990).

Results

The restriction fragments generated by the 23 enzymes are listed in Table 1. The initial survey using 12 restriction enzymes produced 191 detectable restriction sites in 37 brown trout, representing an average of 819.3 nucleotides or 4.9% of the mtDNA genome (calculated according to Nei 1987). Five restriction enzymes were informative: *Ava*II, *Hae*III, *Hin*fI, *Mbo*I and *Xba*I. No genome length variation or heteroplasmy was observed. Seven mtDNA haplotypes were detected in the initial sample of 37 trout. Examination of a further 139 trout with the informative enzymes revealed only one additional haplotype (VIII). The frequencies of all eight haplotypes observed in the total sample are given in Table 2. Of the five informative restriction enzymes only two (*Xba*I and *Mbo*I morph A <-> B)

Table 1. mtDNA restriction fragments generated in *Salmo trutta* L. individuals from ferox, sonaghen, and gillaroo from Lough Melvin, northwest Ireland.

Enzyme	Restriction morph	Restriction fragment sizes (in bp)	Total size
*Ava*II	A	4030, 2825, 2637, 2204, 1427, 807, 433, 300, 292, 192, 181	15 328
	B	+4675, −2825, −2204, +465	15 440
	C	−2204, +1573, +465, +149	15 311
*Bam*HI	A	6419, 5510, 4343, 845	17 117
*Bgl*I	A	4300, 4078, 2734, 2649, 1152, 1103	16 016
*Bgl*II	A	14 746, 862	15 608
*Bsc*I	A	16 881	16 881
*Dde*I	A	1143, 1046, 883, 821, 724, 685, 645, 572, 510, 491, 433, 410, 402, 374, 356, 300, 280, 270, 255, 226, 217, 200, 179, 174, 166, 156, 154, 145, 141, 136, 129, 122, 118, 114, 106, 102, 100, 99, 97, 95, 93, 90, 76, 73	13 908
*Dra*I	A	6380, 5423, 1648, 755, 554	14 760
*Fnu*DII	A	3545, 2581, 1538, 1200, 1136, 642, 551, 500, 496, 395, 287	12 881
*Hae*III	A	1649, 1348, 831, 718, 695, 541, 470, 428, 418, 326, 317, 259, 250, 188	8 438
	B	−695, +578, +502, −418	8 405
*Hha*I	A	6507, 2173, 1810, 1492, 998, 870, 742, 644, 626, 409, 400, 250, 219	17 140
*Hinc*II	A	3565, 2743, 2417, 2011, 1845, 1007, 815, 679, 615, 554	16 251
*Hind*III	A	7926, 3552, 2348, 1799, 838, 250	16 713
*Hin*fI	A	2322, 1241, 1155,* 1054, 1013, 950, 905, 869, 821, 750, 702, 610, 537, 497, 444, 420, 375, 349, 334, 314, 281, 240, 212, 195, 141, 93	16 824
	C	−1054, +932, +352, −141, +93	16 820
	D	−1155,* +892, +352, +145, −141, −93	16 824
	E	−1155,* +1043,* +775, −420, −349, +340, −141, −93	16 824
*Hpa*I	A	11 352, 2166, 1419, 765	15 702
*Hpa*II	A	1457, 1380, 1214, 1067, 762, 746, 699, 685, 663, 549, 530, 518, 488, 459, 371, 335, 324, 314, 265, 251, 208, 168, 159, 137, 121, 114, 101, 70, 67, 65, 52, 42, 38, 37, 35	14 491
*Kpn*I	A	17 119	17 119
*Mbo*I	A	1813, 1377, 1226, 1094, 1051, 943, 907, 715, 689, 595, 574, 475, 460, 420, 280, 271, 222, 163, 151, 105, 91, 83, 72, 71	13 848
	B	+491, −271, −222	13 846
	C	+605, −595, −574, +555	13 839
*Pst*I	A	13 581, 3636	17 217
*Pvu*II	A	13 092, 3796	16 888
*Rsa*I	A	2338, 2016, 1883, 1356, 1147, 1067, 911, 715, 624, 512, 376, 338, 321, 258, 254, 214, 202, 189, 162, 143, 132, 130, 114, 109, 96, 94, 89, 87	15 877
*Sma*I	A	16 470	16 470
*Taq*I	A	2049, 1663, 1257, 1183, 1049, 1019, 803, 729, 656, 632, 595, 521, 444, 402, 375, 302, 263, 222, 214, 184, 142, 122, 117, 86, 76, 67, 61	15 233
*Xba*I	A	8456, 4139, 3512, 2119	18 226
	B	−8456, +6394, +1975	18 139

*Fragments inferred to give the minimum number of restriction site changes between lineages. These were not resolved on the gels.

produced variant patterns that could be explained by single nucleotide substitutions. All other patterns were consistent with multiple substitution events that ranged from two (all *Ava*II changes; *Hae*III A <-> B; *Mbo*I A <-> C) to five (*Hin*fI C <-> E). Nucleon diversity values ranged from 0 in the ferox, where all individuals had the same haplotype, to 0.76 in gillaroo and 0.77 in sonaghen (Table 2).

All ferox surveyed were monomorphic at all restriction sites examined and were assigned to haplotype I. This haplotype was also found in both gillaroo and sonaghen and overall 44% of the trout were of this haplotype. Six haplotypes were found in the gillaroo, three of which (VII, VIII, and IX) were unique to this group. The three gillaroo-specific haplotypes represented 55% of this group. Haplotype VII

Table 2. Frequencies and diversity of mitochondrial DNA haplotypes in Melvin brown trout populations.

			Haplotype								
Population	Year	*N*	I BAAAA	II ABAAA	III BADAA	V ACEAA	VI ACAAA	VII ABAAB	VIII ABAAC	IX ACCBA	Nucleon diversity
Ferox	1986	9	1.0								
	1990	37	1.0								
	Total	46	1.0								0
Gillaroo	1986	9	0.22	0.22	0	0	0.22	0.22	0	0.11	
	1990	47	0.13	0.04	0	0	0.23	0.46	0.06	0.08	
	Total	56	0.14	0.07	0	0	0.23	0.41	0.05	0.09	0.76
Sonaghen	1986	19	0.53	0.05	0.21	0.05	0.16	0	0	0	
	1990	55	0.25	0.29	0.26	0.16	0.04	0	0	0	
	Total	74	0.32	0.23	0.24	0.14	0.07	0	0	0	0.77

Note: Haplotypes comprise *Xba*I, *Ava*II, *Hin*fI, *Hae*III, and *Mbo*I restriction fragment patterns in sequence.

was most similar to haplotype II from which it differed by one *Mbo*I restriction site, whereas haplotype VIII differed from II at two *Mbo*I restriction sites. Haplotype IX differed from VI at a total of five restriction sites; three were due to *Hin*fI and two to *Hae*III restriction site differences. The percent sequence divergence between these unique gillaroo haplotypes and haplotypes II and VI ranged from 0.07 to 0.58.

Five haplotypes were found in the sonaghen, two of which (III and V) were specific to this group and represented 38% of the total fish examined. These sonaghen-specific haplotypes differed from each other at seven restriction sites (*Ava*II, *Hin*fI, and *Xba*I). Haplotype III differed from I at four restriction sites owing to mutations at *Hin*fI sites. Haplotype V differed from VI also at four *Hin*fI sites. A 0.14% sequence divergence existed between haplotypes III and I and between V and VI. The remaining two haplotypes that were shared by both sonaghen and gillaroo were also present at quite different frequencies (see Table 2).

AMOVA revealed significant differences among the three types ($P = 0.0210$, 1000 permutations), while no significant differences were observed between year-classes within types ($P = 0.0669$, 1000 permutations), indicating that there was temporal stability. A θ value of 0.334 was calculated for the three types overall.

Discussion

MtDNA variability in brown trout

This study represents the most extensive RFLP survey of *S. trutta* populations reported to date, where 176 trout were examined with five informative enzymes. These produced polymorphisms not previously described including *Xba*I, *Hin*fI, and *Mbo*I variants. Gyllensten and Wilson (1987) described *Ava*II and *Hae*III variation within *S. trutta* populations and Hynes et al. (1989) described three *Hin*fI variants. In this study four *Hin*fI variants are described. Variation at *Xba*I sites was not detected in 9 brown trout from Sweden (Gyllensten and Wilson 1987) nor in 27 brown trout from Tasmania (Ovenden et al. 1993). It is not possible to directly compare the variants from *Hae*III and *Ava*II described here with those from previous studies without running side by side comparisons. Two *Mbo*I variants were originally described in gillaroo by McVeigh and Ferguson (1988). In this extended study a third variant was discovered and again this was unique to gillaroo.

The extensive RFLP variation found in the mtDNA of the Melvin trout samples contrasts with the absence of polymorphism in brown trout as revealed by sequencing studies. Bernatchez et al. (1992) sequenced 640 bp of the mitochondrial control region and found it to be invariant. Similarly, partial sequences of the cytochrome *b* gene of Melvin and other brown trout populations revealed no variation (H.P. McVeigh, unpublished data). Low levels of sequence variation were discovered in sections of the cytochrome *b* and ATPase 6 genes in brown trout populations in Italy (Giuffra et al. 1994). The latter differences in sequence were all due to transitions, mostly at third codon positions, only one of which in the ATPase 6 gene gave rise to a conservative amino acid replacement.

It is of interest to note that in our study only one additional haplotype was found when a further 139 fish were examined compared with the original sample of 37, although the frequency estimates did change substantially. However, unique haplotypes are present in both the 1986 and 1990 samples of gillaroo and sonaghen, confirming that the differences between these types are not the result of sampling error or natural stochastic processes.

The lack of variability in ferox mtDNA is in keeping with the reduced heterozygosity found in this type at both allozyme and minisatellite loci. The mean allozyme expected heterozygosity value (46 loci) for ferox was calculated at 0.038 compared with equivalent values of 0.066 for gillaroo and 0.072 for sonaghen (Ferguson and Taggart 1991). Mean heterozygosity based on five minisatellite loci was 0.53 for ferox, 0.62 for gillaroo, and 0.60 for sonaghen (Prodöhl 1993). The reduced variation within the ferox population is probably the result of a reduced effective population size. Ferox represent less than 3% of the total trout numbers in Lough Melvin and have a restricted spawning

area in a single river (Ferguson and Taggart 1991). Bottleneck events have a more drastic effect on mtDNA variation than on the nuclear genome owing to the reduced effective population size of the mitochondrial genome (1/4 assuming equal numbers of males and females).

Genetic differentiation of brown trout types

The occurrence of three unique mtDNA haplotypes in gillaroo and two in sonaghen, coupled with the significant frequency differences in shared haplotypes among all groups, adds further convincing evidence that the three types are genetically distinct. This confirms and extends the information already available from allozyme and minisatellite DNA studies. In particular, the unique nature of gillaroo is confirmed by the presence of unique haplotypes, one at high frequency. This result is congruent with allozyme data where the *LDH-1*QO* allele was only found in gillaroo, being present at a frequency of 0.17. Several other loci showed significant differences in frequency among the types (Ferguson and Taggart 1991). A similar pattern was seen for five minisatellite DNA loci (Prodöhl 1993). At these loci from three to eight alleles were found in the three types but the highest frequency for a unique allele was 0.09. Given the large number of alleles at each locus and sample sizes of 50 to 150, these latter occurrences are likely to represent sampling artifacts. Significant differences in frequency were again found at all loci. Thus mtDNA, with the highest frequency of a unique haplotype being 0.41 in gillaroo and 0.24 in sonaghen, provides the most discriminating marker system. The θ value of 0.334 confirms the genetic heterogeneity of the three types and is comparable with F_{ST} estimates of 0.17 and 0.148 obtained from isozyme and minisatellite data, respectively. The mtDNA value is approximately twice that of those obtained from isozyme and minisatellite studies as would be expected for a haploid genome.

Origins

Two hypotheses have been advanced to explain the origin of the Melvin ferox, gillaroo, and sonaghen. The occurrence of these sympatric, reproductively isolated trout populations could be the result of divergence of allopatric forms that colonized the lake independently. Alternatively they could be the result of sympatric splitting within the lake from one or more postglacial ancestors. It has been suggested previously (Hamilton et al. 1989) that there were two postglacial colonizations of northwestern Europe by brown trout. The first, or ancestral, race is today represented by ferox and the second colonization was by a later, modern race, which was the ancestor of gillaroo and sonaghen. On the basis of morphological and allozyme data, it has been suggested (Cawdery and Ferguson 1988) that gillaroo and sonaghen are the result of a sympatric splitting within Lough Melvin. Together gillaroo and sonaghen have five unique mtDNA haplotypes out of a total of eight and an overall sequence divergence of 1.24% was observed between gillaroo and sonaghen. Such a degree of divergence is unlikely to have arisen within the 13 000 years since the postglacial colonization of Melvin. Thus, if the rate of mtDNA evolution is taken as 2% per million years (Brown et al. 1979) then 0.4 base pair substitutions (0.05%), in the proportion of the genome examined, would be expected between two completely isolated populations in this time. This would suggest that the two types differentiated at a much earlier time and independently colonized Melvin. An alternative explanation is that a single gillaroo–sonaghen ancestor possessing all eight mtDNA haplotypes colonized Melvin postglacially. Stochastic haplotype sorting during bottlenecks in the subsequent divergence of the two types may have resulted in the loss of different haplotypes in the two types. Confirmation of the origins of Melvin ferox, sonaghen, and gillaroo will require examination of more brown trout populations from the Atlantic basin and the construction of an accurate phylogeny of the mitochondrial haplotypes. Such a study is currently in progress.

Status

The confirmation of the reproductive isolation of the ferox, sonaghen, and gillaroo and the provision of additional high resolution characters by which they can be differentiated support the suggestion (Ferguson and Taggart 1991) that they should be classified as distinct subspecies. Thus, ferox would be designated as *Salmo trutta ferox* Jardine and Selby 1835, gillaroo as *Salmo trutta stomachius* Gunther 1866, and sonaghen as *Salmo trutta nigripinnis* Gunther 1866. A prerequisite to conservation is the identification of phylogenetically distinct entities (Utter et al. 1993). Thus, recognition of the Melvin populations as separate subspecies highlights the considerable genetic, behavioural, and ecological differences among the three types and hence the need for appropriate management and conservation action.

Acknowledgement

This research was supported by a studentship from the Department of Agriculture for Northern Ireland to H.P.McV.

References

Bernatchez, L., and J.J. Dodson. 1991. Phylogeographic structure in mitochondrial DNA of the lake whitefish (*Coregonus clupeaformis*) and its relation to Pleistocene glaciations. Evolution, 45: 1016–1035.

Bernatchez, L., R. Guyomard, and F. Bonhomme. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. Mol. Ecol. 1: 161–173.

Billington, N., and P.D.N. Hebert. 1988. Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. Can. J. Fish. Aquat. Sci. 45: 643–654.

Brown, W.M., M. George, Jr., and A.C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. 76: 1967–1971.

Cawdery, S.A.H., and A. Ferguson. 1988. Origins and differentiation of three sympatric species of trout (*Salmo trutta*, L.) in Lough Melvin, Ireland. Pol. J. Hydrobiol. 35(Suppl. A): 267–277.

Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131: 479–491.

Ferguson, A. 1986. Lough Melvin: a unique fish community. *In* Occasional papers in Irish science & technology. No. 4. Royal Dublin Society, Ireland.

Ferguson, A., and F.M. Mason. 1981. Allozyme evidence for reproductively isolated sympatric populations of brown trout, *Salmo trutta* L. in Lough Melvin, Ireland. J. Fish Biol. 18: 629–642.

Ferguson, A., and J.B. Taggart. 1991. Genetic differentiation among the sympatric brown trout (*Salmo trutta*) populations of Lough Melvin, Ireland. Biol. J. Linn. Soc. 43: 221–237.

Giuffra, E., L. Bernatchez, and R. Guyomard. 1994. Mitochondrial control region and protein coding genes sequence variation among phenotypic forms of brown trout *Salmo trutta* from northern Italy. Mol. Ecol. 3: 161–171.

Gyllensten, U., and A.C. Wilson. 1987. Mitochondrial DNA of salmonids: inter- and intraspecific variability selected with restriction enzymes. *In* Population genetics and fishery management. *Edited by* N. Ryman and F. Utter. University of Washington Press, Seattle. pp. 301–317.

Hamilton, K.E., A. Ferguson, J.T. Taggart, T. Tomasson, A. Walker, and E. Fahy. 1989. Post glacial colonisation of brown trout *Salmo trutta* L: LDH as a phylogeographic marker locus. J. Fish Biol. 35: 651–664.

Hynes, R.A., E.J. Duke, and P. Joyce. 1989. Mitochondrial DNA as a genetic marker for brown trout, *Salmo trutta* L., populations. J. Fish Biol. 35: 687–701.

McVeigh, H.P. 1989. Mitochondrial DNA and salmonid population structure. Ph.D. thesis, The Queen's University of Belfast. British Library Microfilm No. DX89773.

McVeigh, H.P., and A. Ferguson. 1988. The applications of mitochondrial DNA in the study of the population biology of the brown trout, *Salmo trutta* L. Pol. J. Hydrobiol. 35(Suppl. A): 287–294.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., and W.-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. 76: 5269–5273.

Nei, M., and F. Tajima. 1981. DNA polymorphism detected by restriction endonucleases. Genetics, 97: 145–163.

Ovenden, J.R., R. Bywater, and R.W.G. White. 1993. Mitochondrial DNA nucleotide sequence variation in Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) from Tasmania, Australia. Aquaculture, 114: 217–227.

Palva, T.K. 1986. Cytogenetic and mitochondrial DNA analysis of four salmonid fish species. Ph.D. thesis, University of Kuopia, Finland.

Prodöhl, P.A. 1993. Multilocus and single locus minisatellite polymorphism in brown trout (*Salmo trutta* L.) populations. Ph.D. thesis, The Queen's University of Belfast.

Prodöhl, P.A., J.B. Taggart, and A. Ferguson. 1992. Genetic variability within and among sympatric brown trout (*Salmo trutta*) populations: multi-locus DNA fingerprint analysis. Hereditas, 117: 45–50.

Taggart, J.B., R.A. Hynes, P.A. Prodöhl, and A. Ferguson. 1992. A simplified protocol for routine total DNA isolation from salmonid fishes. J. Fish Biol. 40: 963–965.

Utter, F.M., J.E. Seeb, and L.W. Seeb. 1993. Complementary uses of ecological and biochemical genetic data in identifying and conserving salmon populations. Fish. Res. 18: 59–76.

Weir, B.S. 1990. Genetic data analysis. Sinauer Associates, Inc., Sunderland, Mass.

Effect of aluminium and lead on activity in the freshwater pond snail *Lymnaea stagnalis*

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Abstract: Time-lapse video was used to examine the effect of short- (19 h) and long-term (1 year) exposure to Al or Pb at neutral pH in static water conditions on the total distance moved by the freshwater snail *Lymnaea stagnalis*. Aluminium at 100, 200, 500, or 1000 $\mu g \cdot L^{-1}$ normally depressed and Pb at 50 and 200 $\mu g \cdot L^{-1}$ often increased activity over the first 19 h of exposure. Smaller (younger) snails generally showed greater sensitivity to Al. Exposure to 100 or 500 $\mu g\ Al \cdot L^{-1}$ for up to 30 days caused hyperactivity, but thereafter and 1 year later, activity was similar to controls, suggesting that the snails had become tolerant. Lead (200 $\mu g \cdot L^{-1}$) caused continued hyperactivity for up to 50 days, although movement was significantly reduced after 1 year, suggesting that acclimation had occurred. The effect of Al is interesting given the supposed limited bioavailability of this metal at neutral pH.

Résumé : On a appliqué la technique d'enregistrement vidéo accéléré à l'examen de l'effet d'une exposition à court terme (19 h) et à long terme (1 an) du gastropode d'eau douce *Lymnaea stagnalis* au Al et au Pb à pH neutre, effet mesuré par la distance totale franchie par les sujets dans des bassins sans circulation d'eau. À 100, 200, 500 ou 1000 $\mu g \cdot L^{-1}$, l'aluminium exerçait normalement un effet dépresseur alors qu'à 50 et 200 $\mu g \cdot L^{-1}$, le plomb exerçait un effet stimulant sur l'activité des sujets pendant les 19 premières h d'exposition. Les gastropodes plus jeunes et plus petits se sont révélés être généralement plus sensibles à l'aluminium. Jusqu'à 30 jours, l'exposition à 100 ou 500 $\mu g \cdot L^{-1}$ d'Al conduisait à l'hyperactivité, mais par la suite, et encore au bout d'un an, l'activité mesurée était semblable à celle des témoins; cela paraît indiquer que les gastropodes acquéraient une tolérance à ces éléments. Le plomb (200 $\mu g \cdot L^{-1}$) provoquait une hyperactivité soutenue pendant 50 jours, mais les déplacements s'étaient significativement réduits après 1 an, ce qui laisse supposer qu'il s'est produit une acclimatation. L'effet de l'aluminium est intéressant en ce qu'on présume que sa biodisponibilité est réduite à pH neutre.

[Traduit par la Rédaction]

Introduction

Freshwater macroinvertebrates are known to accumulate Al and Pb from road runoff, acidification of the surrounding catchment, and mining (McCrae 1988; McCahon et al. 1989; Pynnönen 1990; Madigosky et al. 1991). Aluminium also enters river systems following treatment of drinking water and sewage with aluminium sulphate, though it is thought to have negligible environmental impact at neutral pH (Furr and Mooney 1971).

Although some of the physiological mechanisms underlying the toxicity of Al and Pb are known (Muniz and Leivestad 1980), the effect of these metals on the behaviour of whole animals is poorly documented. Behavioural changes induced by pollutants are important because they can lead to reduced survival and reproductive success. Behaviour may also be a more sensitive indicator of toxicity than ultrastructural change (Norton 1978). The use of animals with a restricted behavioural repertoire in studies examining the effect of toxicants on behaviour allows results to be more readily interpreted without complicating behavioural interactions (Norton 1978; Tilson and Cabe 1978). The freshwater pulmonate *Lymnaea stagnalis*, a browsing omnivore, fulfills this criterion and its behaviour and physiology have been widely studied (Tuersley and McCrohan 1987; McCrohan et al. 1989). Locomotion and its neuronal basis have also been examined in *L. stagnalis* (Kaiser 1960; Winlow and Haydon 1986; McKenzie et al. 1987; Syed and Winlow 1989). This species is therefore a potential model system in which to examine the effects of Al and Pb on activity and, ultimately, on the underlying physiology of the nervous system.

This paper examines the effect of continuous exposure to Al and Pb over the short (19 h) and long term (1 year) on total distance moved by *L. stagnalis*, using time-lapse video. The effects of Al and Pb on snails of different age and size were assessed. The influence of temperature and intraspecific interactions on activity was also examined. Finally, to determine whether tolerance to Al and Pb occurs, activity was examined over a 50-day exposure, and in *L. stagnalis* reared in Al or Pb for 1 year.

Received June 18, 1994. Accepted March 3, 1995.
J11973

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Material and methods

Lymnaea stagnalis were obtained from animal suppliers (Blades, Kent, U.K., or Sciento, Salford, U.K.) and maintained in aerated standard snail water (SSW) (Thomas et al. 1975) for 10–14 days before experimentation. Snails were fed lettuce throughout and maintained under a 12 h light : 12 h dark regime.

Aluminium and Pb were added (as nitrate) from stock solutions made up in distilled water. Total Al concentrations were monitored with a Skalar autoanalyzer (Skalar analytical BV, The Netherlands) using a colourimetric method that measures absorbance at 580 nm following the pyrocatechol violet reaction (Her Majesty's Stationery Office 1987). Total Pb levels were not monitored. The pH ranged between 6.6 and 7.2 ($n = 80$).

Measurement of movement

Snail movements were recorded at a rate of 1 frame/s by an infrared video camera (Panasonic, model WV-1850/B/ER) fitted with a Computar APC wide-angle lens (f12.5 mm) and connected to a time-lapse video recorder (Panasonic, model AG-6010). An infrared light source enabled recording during darkness. Recordings were replayed at normal speed and a movement profile was obtained for each snail by manually tracing its position onto an acetate sheet placed over the video monitor. The total distance moved by each snail was calculated from the trace using a sonic digitizer connected to a microcomputer.

Movements were normally recorded from snails kept individually in small arenas (171 × 113 mm) containing 300 mL of unaerated SSW. This restricted vertical movement, so activity was recorded in two dimensions. To determine whether interactions between individuals affected movement, snails in groups of two or three were recorded in larger arenas (155 × 190 mm, containing 2.5 L of SSW). Experiments 1 and 2 were carried out at a temperature of 15 ± 1°C; however, it was found that activity was greater at 20 ± 1°C. Therefore, later experiments were carried out at 20 ± 1°C to determine the effect of Al and Pb exposure on more active snails.

Experiment 1 investigated the effect of Al and Pb exposure on locomotory activity over 50 days. Large adult snails (>30 mm shell length) were exposed to 0 (control), 100, or 500 $\mu g \cdot L^{-1}$ added Al, or 200 $\mu g \cdot L^{-1}$ added Pb at 15 ± 1°C during April and May 1991. Immediately after initial exposure, 10 snails from each treatment were placed individually in small arenas, and following a 1-h acclimatization period, their activity was recorded for 18 h. Every week for 7 weeks, movements were similarly recorded for 2 h, between 8:00 and 10:00 a.m. (the time of day when controls were most active). The water was changed weekly following a recording session.

Experiment 2 was conducted during August 1991. Smaller adults (20–25 mm shell length) were exposed for 19 h to 0 (control), 100, or 500 $\mu g \cdot L^{-1}$ added Al, or 50 $\mu g \cdot L^{-1}$ added Pb at 15 ± 1°C. Movements of 12 snails/treatment were recorded in groups of either 2 or 3 in large arenas.

Experiment 3 was conducted during November 1991. Ten juveniles (10–15 mm shell length) in each treatment were kept individually in small arenas at 20 ± 1°C and their activity was recorded over the first 45 h immediately following exposure to 0, 100, 200, 500, or 1000 $\mu g \cdot L^{-1}$ added Al.

Experiment 4 examined the response of snails of different sizes to Al and young snails to Pb over 19 h at 20 ± 1°C. Ten juveniles (15–20 mm shell length) and 10 large adults (27–34 mm shell length) were exposed to SSW only (controls) or 100 $\mu g \cdot L^{-1}$ added Al, and 10 juveniles (15–20 mm shell length) were exposed to 50 $\mu g \cdot L^{-1}$ added Pb. Movements were recorded from individuals in small arenas during May 1992.

In experiment 5, to determine whether tolerance to Al and Pb occurs, eggs laid in experiment 3 were collected for each treatment and hatched snails reared in their respective metal concentrations for 1 year under 12 h light : 12 h dark conditions. Movements of 10 snails exposed to 0, 100, or 500 $\mu g \cdot L^{-1}$ added Al, or 200 $\mu g \cdot L^{-1}$ added Pb, were recorded at 20 ± 1°C for 24 h.

Analysis of results

Distances moved per hour by snails under different treatments were compared using either the Mann–Whitney *U* test or the Kruskal–Wallis one-way analysis of variance by ranks for multiple comparisons (Siegel and Castellan 1988). A significance level of $P < 0.05$ was chosen.

Results

Effect of body size, temperature, and interspecific interactions on total movement by control snails

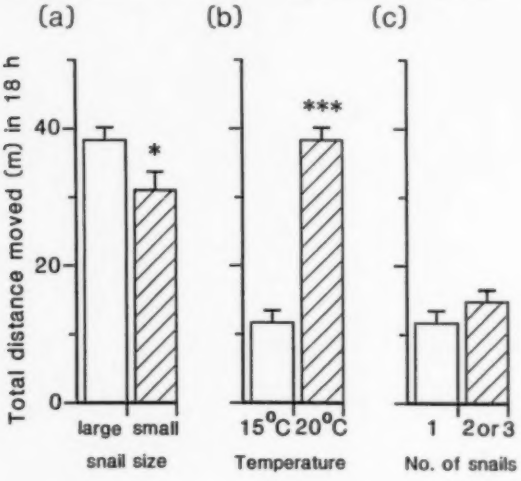
Movement of control snails was highly variable over the whole course of testing and between experiments. Much of the variability was due to differences in experimental design. Snail size is important, as large control snails moved further ($P < 0.05$) than juveniles in experiment 4 (Fig. 1*a*). Movement was also greater ($P < 0.001$) in large snails at 20°C in experiment 4 than in similar snails at 15°C in experiment 1, when both were performed at the same time of year (Fig. 1*b*). However, there was no significant difference in activity at 15°C between snails observed individually (in experiment 1), and in groups of two or three (in experiment 2; Fig. 1*c*). We presume that some variability between experiments is due to seasonal changes.

Effect of exposure to Al on activity

Short-term exposure

With one exception, exposure to Al (100–1000 $\mu g \cdot L^{-1}$) reduced activity in the first three experiments (Figs. 2 and 3). A concentration of 100 $\mu g \cdot L^{-1}$ Al significantly increased the activity of adults compared with controls in experiment 1 after 9 and 10 h exposure only (Fig. 2*a*). The time at which significant reductions in activity occurred varied for different Al concentrations and between experiments. Aluminium at 500 $\mu g \cdot L^{-1}$ significantly reduced activity in large adult snails after 5 and 6 h exposure in experiment 1 (Fig. 2*a*); after 4 h in adults in experiment 2, where activity remained significantly depressed for the following 11 h (Fig. 2*b*); and after 4 h in juveniles in experiment 3, where activity was significantly reduced for the remainder of the period (Fig. 2*c*). Aluminium at 100 $\mu g \cdot L^{-1}$ significantly reduced activity in adults after

Fig. 1. Total distance moved in 18 h by control snails. (*a*) Adults (open column) and juveniles (hatched column) from experiment 4. (*b*) Adults kept at 20°C from experiment 4 (hatched column) and similarly sized snails from experiment 1 maintained at 15°C (open column). (*c*) Adults recorded individually from experiment 1 (open column) and in groups of 2 or 3 from experiment 2 (hatched column). Error bars indicate ±1 SE. *, significant at $P < 0.05$; ***, significant at $P < 0.001$.

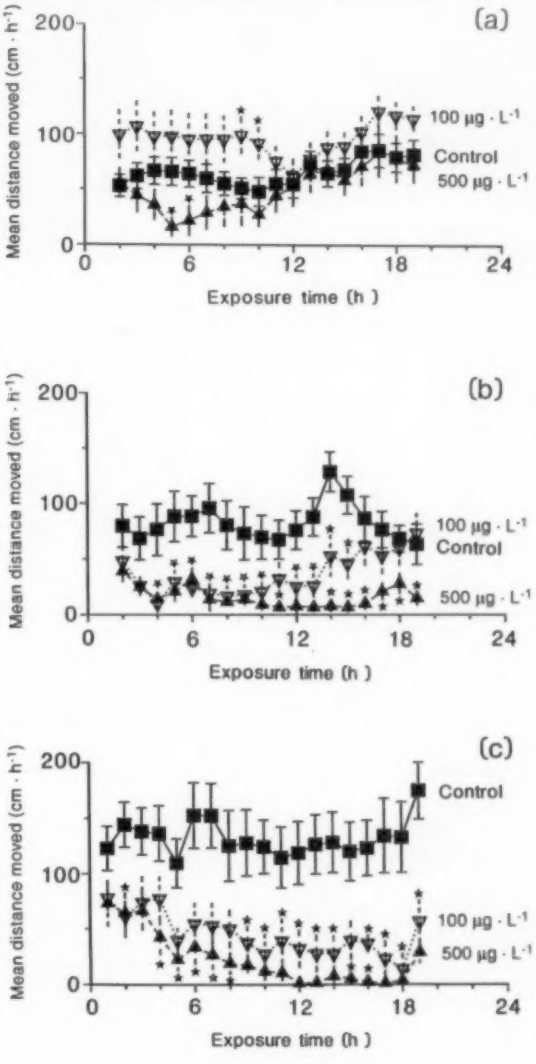


4 h exposure and for the next 10 h in experiment 2 (Fig. 2*b*). Juveniles exposed to 100 μg Al·L^{-1} in experiment 3 showed significantly reduced movements after 4 h exposure and remained significantly less active (Fig. 2*c*). A clear concentration effect of Al on activity was shown by juveniles. Activity was significantly reduced after only 2 h in 1000 μg Al·L^{-1}, 4 h in 500 μg Al·L^{-1}, and 10 h in 100 and 200 μg Al·L^{-1} (Figs. 2*c* and 3).

To determine whether snail activity remained depressed, movement by juveniles was measured over 45 h in controls and in snails exposed to 200 μg Al·L^{-1} only (Fig. 3). The mean distance moved per hour fluctuated in both groups, and these variations suggest the presence of an activity rhythm. The maximum activity occurred between 10:00 a.m. and midday, with minimal movements around midnight. Activity was depressed on exposure to 200 μg Al·L^{-1} throughout the rhythm.

The differences observed between experiments 1, 2, and 3 suggest that juveniles are more sensitive to Al than adults. Experiment 4 was designed to examine this further by quantifying the effect of exposure to 100 μg Al·L^{-1} for 19 h on activity in two size-classes of snail. In large adults, activity was reduced significantly between 10 and 13 h, and after 17 and 18 h exposure (Fig. 4*a*). In juveniles, activity was reduced significantly after 8 and 9 h, and at 18 and 19 h (Fig. 4*b*). The earlier depression of locomotion on exposure to 100 μg Al·L^{-1} supports the idea that younger snails are more affected by Al than adults. However, the results are less convincing in this experiment than those observed in the first series of experiments (experiments 1–3) and may indicate variability in

Fig. 2. Effect of exposure to added Al (0 μg·L^{-1}, ■; 100 μg·L^{-1}, ▽; 500 μg·L^{-1}, ▲) for 18 h on mean distance (±1 SE) moved by (*a*) large adults (shell length >30 mm) from experiment 1, (*b*) adults (shell length 20–25 mm) from experiment 2, and (*c*) juveniles (shell length 10–15 mm) from experiment 3. *, significant at $P < 0.05$.



response by different batches of snails. As the lowest concentration of Al (100 μg·L^{-1}) was used in experiment 4, there is a greater likelihood of a reduced response occurring, owing to variability between snails, than at higher concentrations of Al if 100 μg·L^{-1} is close to the threshold concentration at which a response occurs.

Long-term exposure

In large adults from experiment 1, activity was significantly increased after 8, 22, and 28 days exposure to 100 or 500 μg Al·L^{-1} (Fig. 5*a*). Activity was also significantly

Fig. 3. Effect of exposure to 1000 μg·L^{-1} added Al (▲) for 18 h, and 0 (■) and 200 (◆) μg Al·L^{-1} for 45 h, on mean distance (±1 SE) moved by juveniles (shell length 10–15 mm) from experiment 3.

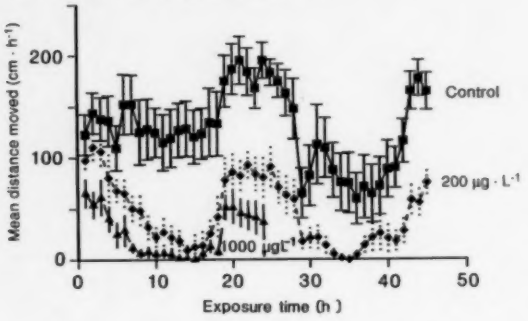
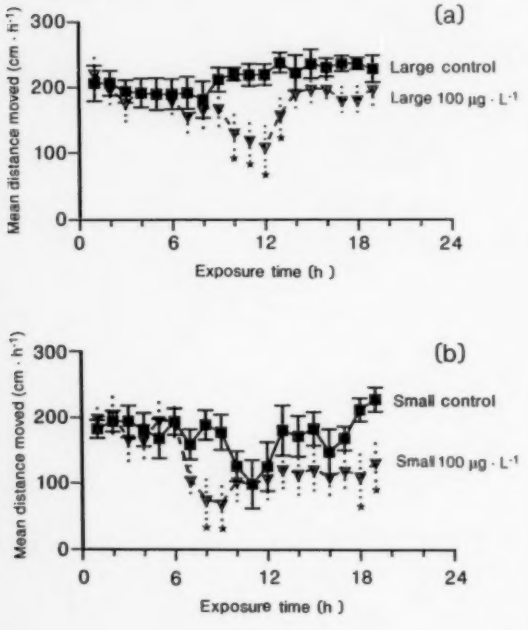


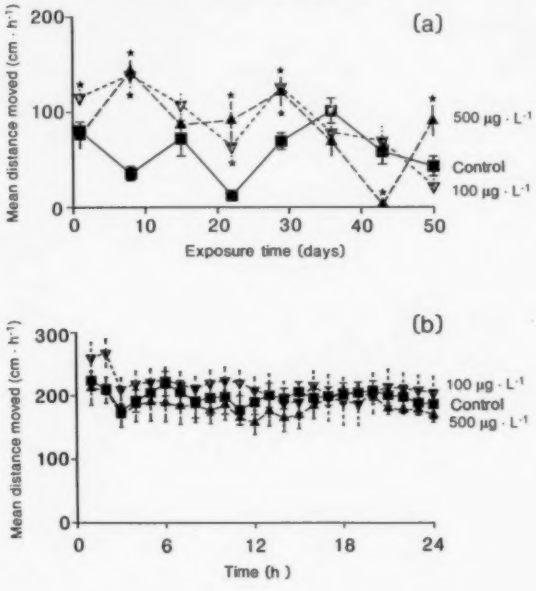
Fig. 4. Effect of exposure to added Al (0 μg·L^{-1}, ■; and 100 μg·L^{-1}, ▽) for 18 h on mean distance (±1 SE) moved by (*a*) adults (shell length 27–34 mm) and (*b*) juveniles (shell length 15–20 mm) from experiment 4. *, significant at $P < 0.05$.



increased after the 1st h of exposure to 100 μg Al·L^{-1}, but not to 500 μg·L^{-1}. Snails exposed to 100 μg Al·L^{-1} for 36, 42, and 50 days showed activity not significantly different from controls. However, exposure to 500 μg Al·L^{-1} over the same period caused significantly reduced activity on day 42 and significant hyperactivity on day 50.

The locomotory activity of snails laid and reared in 0, 100, or 500 μg·L^{-1} added Al for 1 year was measured over 24 h (Fig. 5*b*). Snails from all three treatments showed similar movement and were active throughout 24 h.

Fig. 5. (*a*) Effect of 50 day Al (0 μg·L^{-1}, ■; 100 μg·L^{-1}, ▽; and 500 μg·L^{-1}, ▲) exposure on mean distance (±1 SE) moved per hour between 8:00 and 10:00 a.m. by large adult snails (>30 mm shell length). (*b*) Mean distance (±1 SE) moved by snails laid and reared for 12 months in SSW only (control, ■) or with added Al (100 μg·L^{-1}, ▽; or 500 μg·L^{-1}, ▲). *, significant at $P < 0.05$.



No evidence for a locomotory rhythm was observed in controls or experimental animals.

Effect of exposure to Pb on activity

Short-term exposure

Three experiments examined the short-term effect of Pb on activity in *L. stagnalis*. Large adults in 200 μg Pb·L^{-1} (Fig. 6*a*) and adults in 50 μg Pb·L^{-1} (Fig. 6*b*) showed hyperactivity after exposure for 2, 18, and 19 h. However, large adults showed a significant reduction in activity after exposure for 5 and 6 h to 200 μg Pb·L^{-1} (Fig. 6*a*). Throughout the remainder of the observation period, adults (Fig. 6*b*) and juveniles (Fig. 6*c*) exposed to 50 μg Pb·L^{-1} showed similar activities to controls.

Long-term exposure

Large snails exposed to 200 μg Pb·L^{-1} in experiment 1 remained significantly hyperactive over 50 days exposure (Fig. 7*a*). However, snails reared in 200 μg Pb·L^{-1} for 1 year had a significantly reduced activity over 24 h (Fig. 7*b*).

Discussion

Control snails moved throughout the 12 h light : 12 h dark regime, but activity was highest just prior to midday and lowest around midnight. Snail size and the temperature affected the distance moved. *Lymnaea stagnalis* moves forward by the activity of pedal cilia in mucus (Kaiser

Fig. 6. Effect on mean distance (±1 SE) moved per hour after exposure of (*a*) 200 μg Pb·L^{-1} to adult snails (shell length >30 mm) from experiment 1, (*b*) 50 μg Pb·L^{-1} to adults (shell length 20–25 mm) from experiment 2, and (*c*) 50 μg Pb·L^{-1} to juveniles (shell length 15–20 mm) from experiment 4. *, significant at $P < 0.05$.

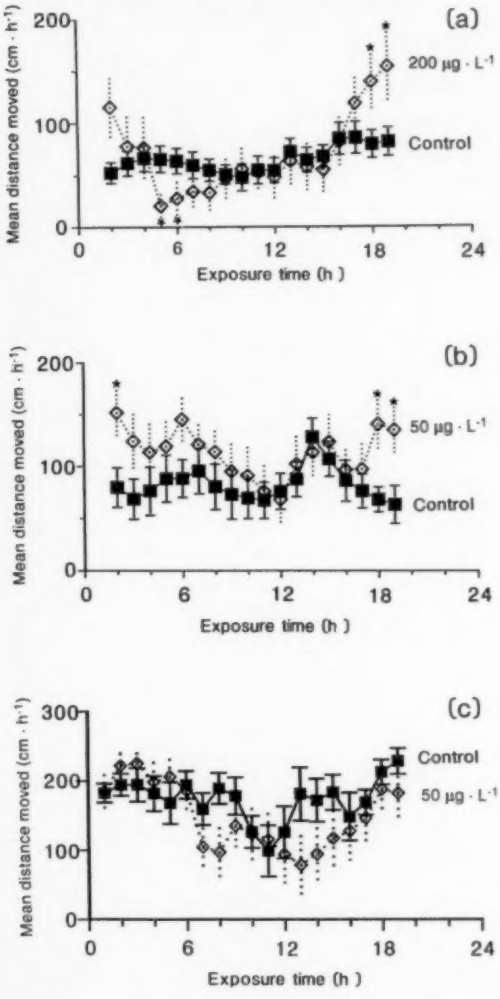
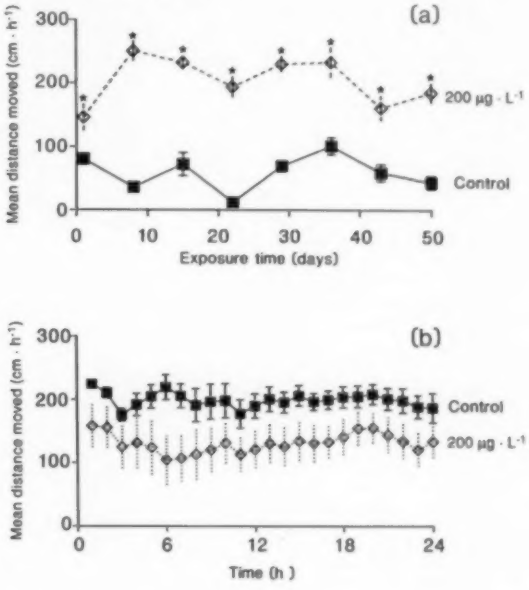


Fig. 7. (*a*) Effect of 50 day Pb (0 μg·L^{-1}, ■; and 200 μg·L^{-1}, ◇) exposure on mean distance (±1 SE) moved per hour between 8:00 and 10:00 a.m. by large adults (>30 mm shell length), and (*b*) mean distance (±1 SE) moved by snails laid and reared for 12 months in SSW only (control, ■) or with added Pb (200 μg·L^{-1}, ◇). *, significant at $P < 0.05$.



1960; Jones 1975). Increased locomotion at 20°C compared with 15°C may be a consequence of increased ciliary activity (Sleigh 1962). Our studies show that there is a trend of reduced activity over short-term exposures to Al, particularly in younger *L. stagnalis*, though the extent of depression varies between batches of snails. This may reflect natural variability, but season, size, and temperature are likely to be important. Lead (50 and 200 μg·L^{-1}) induced hyperactivity in two out of three experiments during the 19 h of initial exposure. Lead (200 μg·L^{-1}) also caused hyperactivity over 50 days of exposure.

The differential response to the metals may reflect their different sites of action and toxicity and may include effects on cilia, mucus production, and the nervous control of cilia and muscles involved in locomotion. However, cilia are not irreversibly damaged when exposed to Al, as snails reared for 1 year in the metal were still able to move. Both ciliary locomotion and movements caused by muscular contraction are under nervous control in *L. stagnalis* (Winlow and Haydon 1986; McKenzie et al. 1987; Syed and Winlow 1989), and it is therefore likely that the nervous system is affected by exposure to Al and Pb. Lead accumulates in ganglia of freshwater snails, causing morphological and biochemical dysfunction (Bolognani et al. 1985), and it modifies the excitability and chemosensitivity of *L. stagnalis* neurons in vitro (Rózsa and Salánki 1990; Salánki et al. 1991). Behavioural changes following exposure to Pb are therefore to be expected. Aluminium is neurotoxic in mammals and in humans it has been implicated in Alzheimer's disease (Liss 1980). Further work is in progress to determine whether the observed differences in locomotory activity in *L. stagnalis* during Al exposure are accompanied by morphological change in the nervous system.

Smaller (younger) snails showed a greater reduction in locomotion, which continued for longer, following exposure to Al. We suggest that this may be due to greater absorption of Al resulting from a greater surface area to volume ratio, and a higher metabolic rate in juveniles, or both. Boyden (1974) found that smaller marine molluscs contained higher concentrations of metal. The decreased effect of Al on activity in large *L. stagnalis* may be due to them being senescent and less responsive to stimuli (Audesirk et al. 1982; Frolkis et al. 1984).

A strategy for minimizing the uptake and toxicity of contaminants is to depress activity, as was observed following exposure of Al in the short term and to Pb after 1 year. Metal uptake is reduced at a lower metabolic rate (Denton and Burdon-Jones 1981) and this may allow survival until more favourable conditions prevail. However, immobility may also indicate sickness. Aluminium exposure over several weeks increased locomotion in *L. stagnalis*, perhaps indicating a switch in strategy to active movement away from the pollutant. Several observations suggest that *L. stagnalis* increases locomotion and movement away from unfavourable conditions including starvation (Scheerboom 1978), and exposure to a chemosterilant (thiourea) and a pesticide (BHC) (Bhide 1989).

Experiments involving snails laid and reared in Al and Pb for 1 year and those exposed to these metals over 50 days suggest that tolerance to Al but not to Pb occurs. Activity was similar to controls in snails exposed to Al for 1 year, but exposure to Pb significantly reduced activity, indicating continuing toxicity. Acclimation to Al has also been observed in rainbow trout (*Salmo gairdneri*) (Orr et al. 1986). Behavioural tolerance to Al after long exposures occurred on constant exposure. However, whether tolerance also occurs during periods of episodic exposure needs to be determined, as this may be more important in the environment (McCahon and Pascoe 1990).

The effect of Al on activity in *L. stagnalis* is much more surprising than that of Pb because Al is thought not to be bioavailable at neutral pH (Martin 1986). Most studies examining the toxicity of Al in freshwater have been done at low pH and with soft water, because of the importance of acid-induced mobilization of Al in fish kills (Haines 1981). However, *L. stagnalis* prefers a water pH of between 6 and 7 (Pullan et al. 1972). In the current study, experiments were performed at neutral pH and with hard water, where 99% of the Al is likely to be in the colloidal hydroxide form ($Al(OH)_4^-$), which is thought not to be bioavailable (Martin 1986). The Al concentrations we used occur in nature; e.g., in circumneutral oligotrophic lakes, Al concentrations reach up to 50 $\mu g \cdot L^{-1}$ (Havas 1986). Our unpublished work on rivers subject to periodic runoff from coal mine wastes shows that they have elevated Al levels (210–800 $\mu g \cdot L^{-1}$) at pH 6.8–7.5, and baseline concentrations greater than 100 $\mu g \cdot L^{-1}$ occur almost continually. The concentrations used in our study were also within the E.C. guidelines (50 μg $Al \cdot L^{-1}$) and maximum admissable concentrations (200 μg $Al \cdot L^{-1}$) in drinking water for human consumption (Johnson and Corcelle 1989). Our preliminary studies show that Al is taken up and accumulated by *L. stagnalis*, with concentration factors of 10^3 when exposed to 100–500 μg $Al \cdot L^{-1}$ for 28 days at neutral pH. Further studies are underway to examine the effect of Al on the snail nervous system. It is envisaged that *L. stagnalis* will provide a good model system to examine the biological effects of Al.

Acknowledgments

We thank Jane Claire for help in analysing the video tapes. This research was carried out during tenure of Natural Environment Research Council research grant GR3/7793.

References

Audesirk, T.E., J.E. Alexander, G.J. Audesirk, and C.M. Moyer. 1982. Rapid, nonaversive conditioning in a freshwater gastropod. I. Effects of age and motivation. Behav. Neural Biol. 36: 379–390.

Bhide, M. 1989. Comparative studies of the effect of Thiourea and BHC on behaviour and mortality in adult snails of the species *Lymnaea stagnalis* and on their egg masses. Folia Morphol. (Prague), 37: 13–18.

Bolognani, F.A.M., A. Franchini, E. Ottaviani, and L. Benedetti. 1985. Effect of pollution on some freshwater species. II. Bioaccumulation and toxic effects of experimental lead pollution on the ganglia in *Viviparus ater* (Mollusca, Gastropoda). Basic Appl. Histochem. 29: 377–387.

Boyden, C.R. 1974. Trace element content and body size in molluscs. Nature (Lond.), 251: 311–314.

Denton, G.R.W., and C. Burdon-Jones. 1981. Influence of temperature and salinity on the uptake, distribution, and depuration of mercury, cadmium, and lead by the black-lip oyster, *Saccostrea echinata*. Mar. Biol. (Berlin), 64: 317–326.

Frolkis, V.V., A.S. Stupina, O.A. Martinenko, S. Toth, and A.I. Timchenko. 1984. Ageing of neurons in the mollusc *Lymnaea stagnalis*. Structure, function and sensitivity to transmitters. Mech. Ageing Dev. 25: 91–102.

Furr, A.K., and E. Mooney. 1971. Neutron activation. Studies of the Roanoke river system. I.E.E.E. Trans. Nucl. Sci. 18: 79.

Haines, T.A. 1981. Acidic precipitation and its consequences for aquatic ecosystems. A review. Trans. Am. Fish. Soc. 110: 669–707.

Havas, M. 1986. Aluminium chemistry of inland waters. *In* Aluminum in the Canadian environment. *Edited by* M. Havas and J.-F. Jaworski. Publ. No. NRCC 24759, National Research Council of Canada, Ottawa, Ont. pp. 51–75.

Her Majesty's Stationery Office. 1987. Methods for the examination of waters and associated materials. Acid soluble aluminium in marine, raw and potable waters. 2nd ed. Her Majesty's Stationery Office, London.

Johnson, S.P., and G. Corcelle. 1989. The environmental policy of the European Communities. Graham and Trotman, London.

Jones, H.D. 1975. Locomotion. *In* Pulmonateso. Vol. 1. *Edited by* V. Fretter and J. Peake. Academic Press, London. pp. 1–32.

Kaiser, P. 1960. Die Leistungen des Flimmerepithels bei der Fortbewegung der Basommatophoran. Z. Wiss. Zool. 162: 368–393.

Liss, L. 1980. Aluminum neurotoxicity. Pathotox Publishers Inc., Park Forest South, Ill.

Madigosky, S.R., X. Alvarez-Hernandez, and J. Glass. 1991. Lead, cadmium and aluminium accumulation in the Red Swamp Crayfish *Procambarus clarkii* G. collected from roadside drainage ditches in Louisiana. Arch. Environ. Contam. Toxicol. 20: 253–258.

Martin, R.B. 1986. The chemistry of aluminum as related to biology and medicine. Clin. Chem. 32: 1797–1806.

McCahon, C.P., and D. Pascoe. 1990. Episodic pollution: causes, toxicological effects and ecological significance. Funct. Ecol. 4: 375–383.

McCahon, C.P., A.F. Brown, M.J. Poulton, and D. Pascoe. 1989. Effects of acid, aluminium and lime additions on fish and invertebrates in a chronically acidic Welsh stream. Water Air Soil Pollut. 45: 345–359.

McCrae, I.S. 1988. Traffic related pollutants, their effects and analytical assessment techniques. Rep. No. R10, Centre for Urban Pollution Research, Middlesex Polytechnic, U.K.

McCrohan, C.R., M.A. Kyriakides, and M.D. Tuersley. 1989.

Initiation and modification of rhythmic buccal motor output in the isolated central nervous system of *Lymnaea stagnalis*. J. Molluscan Stud. 55: 183–192.

McKenzie, J.D., N.I. Syed, J. Tripp, and W. Winlow. 1987. Innervation of ciliated cells and mucus-containing cells in *Lymnaea*. J. Physiol. (Lond.), 386: 32.

Muniz, I.P., and H. Leivestad. 1980. Toxic effects of aluminium on the brown trout, *Salmo trutta*. *In* Ecological Impact of Acid Precipitation: Proceedings of an International Conference, Sandefjord, Norway, March 11–14, 1980. *Edited by* D. Drablos and A. Tollan. SNSF project, Oslo.

Norton, S. 1978. Is behaviour or morphology a more sensitive indicator of central nervous system toxicity? Environ. Health Perspect. 26: 21–27.

Orr, P.L., R.W. Bradley, J.B. Sprague, and N.J. Hutchinson. 1986. Acclimation induced changes in toxicity of aluminium to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 43: 243–246.

Pullan, N.B., F.M. Clino, and C.B. Mansfield. 1972. Studies on the distribution and ecology of the family Lymnaeidae (Mollusca: Gastropoda) in New Zealand. J. R. Soc. N.Z. 2: 393–405.

Pynnönen, K. 1990. Aluminium accumulation and distribution in the freshwater clams (Unionidae). Comp. Biochem. Physiol. C, 97: 111–117.

Rózsa, K.S., and J. Salánki. 1990. Heavy metals regulate physiological and behavioural events by modulating ion channels in neuronal membranes of molluscs. Environ. Monit. Assess. 14: 365–375.

Salánki, J., O.N. Osipenko, T. Kiss, and Györi, J. 1991. Effect of Cu^{2+} and Pb^{2+} on membrane excitability of snail neurons. *In* Molluscan neurobiology. *Edited by* K.S. Kits, H.H. Boer, and J. Joosse. North-Holland, Amsterdam. pp. 214–220.

Scheerboom, J.E.M. 1978. The influence of food quantity and food quality on assimilation, body growth and egg production in the pond snail *Lymnaea stagnalis* (L.) with particular reference to the haemolymph-glucose concentration. Proc. K. Ned. Akad. Wet. Ser. C Biol. Med. Sci. 81: 173–183.

Siegel, S., and N.J. Castellan, Jr. 1988. Nonparametric statistics for the behavioral sciences. 2nd ed. McGraw-Hill Book Co., Maidenhead, U.K.

Sleigh, M.A. 1962. The biology of cilia and flagella. Pergamon Press, Oxford.

Syed, N.I., and W. Winlow. 1989. Morphology and electrophysiology of neurons innervating the ciliated locomotor epithelium in *Lymnaea stagnalis* (L.). Comp. Biochem. Physiol. A, 93: 633–644.

Thomas, J.D., A.S. Lough, and R.W. Lodge. 1975. The chemical ecology of *Biomphalaria glabrata* (Say), the snail host of *Schistosoma mansoni* (Sambon): the search for factors in media conditioned by snails which inhibit their growth and reproduction. J. Appl. Ecol. 12: 421–436.

Tilson, H., and P. Cabe. 1978. Strategy for the assessment of neurobehavioral consequences of environmental factors. Environ. Health Perspect. 26: 287–299.

Tuersley, M.D., and C.R. McCrohan. 1987. Food arousal in the pond snail, *Lymnaea stagnalis*. Behav. Neural Biol. 48: 222–236.

Winlow, W., and Haydon, P.G. 1986. A behavioural and neuronal analysis of the locomotory system of *Lymnaea stagnalis*. Comp. Biochem. Physiol. A, 83: 13–21.

Effects of cadmium on the foraging behavior and growth of juvenile bluegill, *Lepomis macrochirus*[1]

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Abstract: Standardized test protocols for assessing chemical hazards to aquatic organisms inadequately consider behavioral effects of toxicants; yet, organisms behaving abnormally in the wild have reduced growth, reduced fitness, and high mortality. We determined the chronic effects of cadmium (0, 30, 60, 120, and 240 $\mu g \cdot L^{-1}$) on juvenile bluegill (*Lepomis macrochirus*) foraging behavior and growth rates in functional response experiments, each using different sized *Daphnia* as prey. Bluegill consumption rate increased with prey density. Cadmium-exposed fish initially attacked fewer prey per unit of time than unexposed fish, with subsequent recovery to control-level consumption rates determined by cadmium concentration and prey size. The degree of change (over time) in the number of *Daphnia* attacked per 30 s was the most consistently sensitive behavioral measure of sublethal stress in exposed bluegill; the lowest observed effect concentration (LOEC) was 37.3 $\mu g\ Cd \cdot L^{-1}$. Effects on prey attack rates (attacks/30 s) were inversely related to prey size; cadmium had the greatest effect on bluegill foraging on the smallest prey. Cadmium had no effect on prey capture efficiency or handling time. Growth in bluegill length and weight was reduced ($P \leq 0.019$) by all cadmium concentrations and was a more sensitive end point than were the foraging behaviors.

Résumé : Les protocoles d'essai normalisés pour déterminer les risques chimiques chez les organismes aquatiques tiennent insuffisamment compte des effets des substances toxiques sur le comportement. Or, on observe que dans la nature, les organismes manifestant un comportement anormal ont une croissance réduite et sont moins bien ajustés, et leur survie est compromise. Nous avons déterminé les effets chroniques du cadmium (à 0, 30, 60, 120 et 240 $\mu g \cdot L^{-1}$) sur la façon de s'alimenter et sur le taux de croissance de juvéniles du crapet arlequin, *Lepomis macrochirus*, dans des expériences portant sur les réponses fonctionnelles, en prenant des daphnies de tailles différentes comme proies. La consommation s'est accrue avec la densité des proies. Au commencement, les crapets exposés au cadmium attaquaient moins de proies par unité de temps que les sujets non exposés; le rétablissement subséquent jusqu'au niveau de consommation des témoins dépendait de la concentration du cadmium et de la taille des proies. Le dégré de variation (en fonction du temps) du nombre d'attaques sur les daphnies par 30 s, a constitué la mesure la plus régulièrement sensible, sur le plan de comportement d'alimentation, du stress sublétal chez les crapets exposés; la plus faible concentration avec effet observable était de 37,3 $\mu g\ Cd \cdot L^{-1}$. Il existe une relation inverse entre les effets sur la fréquence d'attaque des proies (nombre d'attaques/30 s) et leur taille; l'effet le plus marqué a été observé sur les crapets qui se nourrissaient des plus petites proies. Le cadmium est resté sans effet sur l'efficacité de la capture et le temps de manipulation des proies. On a observé une réduction ($P \leq 0{,}019$) de la croissance en longueur et en poids des crapets exposés au cadmium à toutes les concentrations. C'est là un meilleur résultat final des tests que le comportement d'alimentation.

[Traduit par la Rédaction]

Received April 20, 1994. Accepted March 3, 1995.
J12349

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Introduction

Fish foraging success is largely determined by a complex sequence of behaviors that are influenced by a variety of internal and external factors (Hart 1986). This behavioral sequence includes the motivation to feed and the search patterns used to locate prey, as well as prey identification, pursuit, capture, handling, and ingestion of encountered prey. Foraging success is also affected by characteristics of the prey, including abundance and distribution, species diversity, size and shape, and differential abilities of prey species to escape predation. Toxic substances can affect every aspect of the foraging process through their effects on both predators and prey.

Predator–prey interactions are key linkages between species and trophic levels and are therefore important in controlling community composition and productivity. Understanding and defining the effects of xenobiotics on these interactions is essential in developing a strong hazard assessment protocol (Levin et al. 1984; Sandheinrich and Atchison 1990). However, current assessment of a chemical's potential hazard to aquatic ecosystems relies primarily on standardized acute and chronic laboratory toxicity tests. Such tests only measure physiological effects on growth, survival, and reproduction at the individual and population levels (Maki 1979; Sandheinrich and Atchison 1989). These tests generally fail to consider behavioral effects that are ecologically critical to the well-being of individual organisms, their populations, and communities (Little et al. 1985; Rand 1985; Atchison et al. 1987; Sandheinrich and Atchison 1989, 1990; Beitinger 1990; Henry and Atchison 1991; Scherer 1992).

Behaviors are commonly affected at or below chemical concentrations that alter the physiological end points routinely measured in standardized tests. For example, in their reviews of the effects of metals on fish behavior, Atchison et al. (1987), Sandheinrich and Atchison (1990), and Henry and Atchison (1991) cited numerous studies where avoidance, cough and ventilation rates, social interactions, feeding, and predator avoidance behaviors were significantly altered by metal concentrations near or below the lowest observed effect concentration (LOEC) derived from standardized tests. Although they are sensitive indicators of toxicant exposure, few behaviors have been sufficiently studied to identify their role in growth, survival, or reproduction (Little 1990; Sandheinrich and Atchison 1990). Additional research is needed to identify altered behaviors that affect individual organisms, their populations, and community dynamics within exposed ecosystems. Such research will strengthen our current hazard assessment protocol by providing sensitive, ecologically relevant end points likely to be predictive of real-world effects (Sandheinrich and Atchison 1990).

The purpose of this study was to quantify cadmium-induced changes in foraging behavior that may contribute to altered growth in juvenile bluegill (*Lepomis macrochirus*). Our objectives were (*i*) to compare the sensitivity of traditional end points used to describe fish foraging behavior (e.g., capture efficiency and general motivation to feed) with those that define critical mechanisms of the foraging sequence (e.g., attack, capture, and handling time); (*ii*) to determine the effect of cadmium on bluegill growth; and (*iii*) to compare cadmium concentrations that alter foraging behavior with those that decrease growth, and to evaluate the contribution of altered foraging behavior to the growth effect.

Cadmium was selected as the test chemical because (*i*) it is a common environmental contaminant (Eaton 1974; Kay 1985; Nriagu and Sprague 1987); (*ii*) much is known about its environmental chemistry and toxicological effects on aquatic organisms (Eaton 1974; Gardiner 1974; U.S. Environmental Protection Agency 1985; Eisler 1985); and (*iii*) it alters several fish behaviors at or below the LOEC measured by standardized chronic tests (Atchison et al. 1987; Henry and Atchison 1991).

Methods

Test organisms

The bluegill was chosen as the test species because it (*i*) is a biologically, recreationally, and economically important midwestern species; (*ii*) is commonly used in toxicity testing, and its foraging behavior has been well documented; and (*iii*) has been extensively used in the development of optimal foraging and bioenergetics models. Bluegill, obtained from a private hatchery or collected from a 3-ha pond, were acclimated to laboratory conditions for 1 week prior to stocking into exposure tanks. The total length of bluegill at test initiation was 31.1 ± 1.3 mm (mean ± SD; $n = 180$).

Bluegill and daphnid prey (*Daphnia magna* and *Daphnia pulex*) were reared and tested in dechlorinated (charcoal filtration) tap water with the following characteristics (mean ± SE): temperature, 23.7 ± 0.3°C; pH, 8.0 ± 0.2; dissolved oxygen, 7.4 ± 0.4 mg·L^{-1}; conductivity, 708 ± 33 μmho·cm^{-1} (1 mho = 1 S; $n = 26$); total alkalinity, 141 ± 7 mg·L^{-1} as $CaCO_3$; and total hardness, 174 ± 10 mg·L^{-1} as $CaCO_3$ ($n = 12$).

Functional response experiments

Three functional response experiments, each using a different sized *Daphnia* as prey, were conducted to assess cadmium effects on juvenile bluegill foraging performance. The sizes (mean ± SD; $n = 30$) of *Daphnia* used were large (2.5 ± 0.3 mm), medium (1.5 ± 0.2 mm), and small (0.85 ± 0.15 mm). These sizes were selected to reflect the spectrum of sizes of zooplanktivorous prey that 30-mm bluegill would naturally forage on in the wild. Bluegill were acclimated to exposure tanks on a 16 h light : 8 h dark photoperiod for a minimum of 3 d prior to testing. During this time, all tanks received 2.5 g of frozen brine shrimp (*Artemia salina*) once each day, and fish were allowed to feed for 10 min before all remaining food was removed from tanks by siphoning. In each experiment, 60 bluegill, 6 assigned randomly to each of ten 57-L flow-through tanks (two replicates of five treatments), were used in a proportional diluter system. Nominal exposure concentrations included a control (no added cadmium) and 30, 60, 120, and 240 μg Cd·L^{-1}. American Chemical Society (ACS) certified cadmium chloride ($CdCl_2 \cdot 2\frac{1}{2}H_2O$) was used to establish desired cadmium concentrations in exposure tanks. Each tank received 120 mL·min^{-1} of water.

Fish were identified by cold branding (Everest and Edmundson 1967) so that individuals could be presented with the same prey density throughout the experiment.

Each 26- to 28-d experiment consisted of a 4- to 5-d pretreatment (undosed) period, when the foraging behavior of individual fish was characterized, followed by a treatment (dosed) or control period of 21–22 d when the effects of cadmium were assessed in three 2- to 3-d testing periods. Within an experiment, pretreatment data for each fish were averaged across days to define foraging capabilities prior to cadmium exposure. Cadmium treatments (including the control) began immediately following the last day of pretreatment testing (called time period 1), and foraging trials resumed when cadmium in all exposure tanks reached target concentrations. Data collected on each fish during the first 3 d of cadmium exposure (time period 2) were also averaged to provide mean values for the interval. The foraging parameters assessed in these experiments were again measured for 2–3 consecutive days during the middle (time period 3) and end (time period 4) of the dosed portion of each experiment.

In a foraging trial, an individual fish was removed from its treatment tank and placed into one of three aquaria containing 24 L of control water and either a low (0.5), medium (2.5), or high (15 or 20 prey/L) density of prey. Fish were allowed to forage for 30 s after first prey capture and were then returned to their treatment tank. Experiment 1 used large *Daphnia* as prey, and the highest density was 15 prey/L. Experiments 2 and 3 used medium and small *Daphnia*, respectively, with a high density of 20 prey/L. The total number of prey attacked and captured, capture efficiency (prey captured divided by prey attacked), mean handling time per prey (recorded for trials in high prey density aquaria only) (Sandheinrich and Atchison 1989), and a general assessment of feeding motivation were recorded for each foraging trial. Feeding motivation was assessed by ranking the duration of feeding activity between 0 and 4 (0, fish did not feed; 1, fed for <25% of the allotted 30 s trial; 2, fed for 25–49% of the trial; 3, fed for 50–74% of the trial; 4, fed for 75–100% of the trial).

Following the completion of daily foraging trials, each treatment tank received 2.0 g of frozen brine shrimp and fish were allowed to feed for 8 min before all excess brine shrimp were siphoned from the tank. Bluegill were not fed for approximately 18 h before beginning foraging trials the following day. The order in which tanks were tested was randomized at the start of each day. On days when foraging trials were not conducted, fish were fed once daily; each treatment tank received 2.5 g of frozen brine shrimp, and fish were given 10 min to feed before remaining food was removed by siphoning.

To determine if cadmium affected bluegill growth in experiments 2 (medium *Daphnia*) and 3 (small *Daphnia*), each fish was weighed to the nearest 0.1 mg and measured to the nearest 0.5 mm at test initiation and termination.

Statistical design and analyses

Experiments were a split-plot, completely randomized design with treatments assigned randomly to 57-L tanks (experimental units). The linear statistical model contained cadmium as the whole-plot treatment effect. The subplot contained the effects of prey density and the interactions of cadmium with prey density. The error term used to test for the effect of cadmium (whole-plot) was the mean square error (MSE) for tanks nested within cadmium (F test with 4,5 degrees of freedom (df)). The subplot contained the effects of density (F test with 2,10 df) and cadmium × density interaction (F test with 8,10 df). The error term used to test for the effect of time (F test with 3,15 df) and cadmium × time interaction (F test with 12,15 df) was the MSE for tanks nested within cadmium × time. Cadmium effects on foraging parameters were determined by conducting an analysis of variance on the slopes of the lines generated by regressing the dependent variables against time for each fish. This approach effectively removes the inherent variability among fish treated alike as it analyzes trends over time rather than the raw data values collected at specific points in time. Cadmium effects on bluegill growth in length and weight were also determined by analysis of variance (F test with 4,5 df). For all tests of cadmium effects, the four treatment df were also subdivided into single df contrasts to individually compare the control mean with that of each cadmium treatment (F test with 1,5 df). All analyses of variance were done with the Statistical Analysis System (SAS) general linear models procedure (GLM) (SAS Institute Inc. 1985). Null hypotheses were rejected at $P \leq 0.05$.

Water chemistry and cadmium analyses

Water chemistry analyses were conducted with standard procedures (American Public Health Association, American Water Works Association, and Water Pollution Control Federation 1989; American Society for Testing and Materials 1990). The temperature, pH, conductivity, and dissolved oxygen content of exposure-tank water were measured daily with a Celsius thermometer, Orion model 407A ion meter, Cole Parmer model 1481-50 conductivity meter, and Yellow Springs Instrument (YSI) model 57 oxygen meter, respectively. Oxygen and conductivity meters were calibrated weekly. The pH meter was calibrated daily with buffer solutions of pH 7.0 and 10.0. Total alkalinity and total hardness were measured four times weekly. U.S. EPA quality control samples (1 and 2) for mineral analysis were analyzed along with each batch of water samples to verify procedure accuracy and precision.

Cadmium concentrations in exposure tanks were determined on three 50-mL aliquot samples collected from each tank on two or three occasions during the treatment period. Samples were acidified to pH < 2 with 70% Instra-Analyzed HNO_3 (JT Baker Chemical Co.) and analyzed the same day. Total cadmium concentrations were determined by flame atomic absorption spectrophotometry with an Instrumentation Laboratories model 251 atomic absorption spectrophotometer according to standard methods (American Public Health Association, American Water Works Association, and Water Pollution Control Federation 1989). The method detection limit and the limit of quantification for this analytical procedure were 5.5 and 17.5 $\mu g\ Cd \cdot L^{-1}$, respectively (Taylor 1987). Procedural blanks, calibration standards, spiked samples, and externally supplied quality-assurance samples (Environmental Resource Associates, Arvada,

Colo.) were taken through analytical procedures for each batch of water samples. The certified quality assurance samples were analyzed to assess procedure accuracy. Precision was assessed by analyzing samples in triplicate, while the recovery of cadmium from spiked samples (at least 10% of all samples analyzed) was used to further assure accuracy.

Measured mean exposure concentrations (μg $Cd \cdot L^{-1}$; mean ± SE) for the three experiments conducted were as follows: experiment 1 (large *Daphnia*), below detection limit (control), 26.8 ± 1.1, 61.9 ± 0.6, 121.1 ± 1.2, and 242.6 ± 5.2 (n = 2); experiment 2 (medium *Daphnia*), below detection limit, 37.3 ± 6.4, 64.4 ± 3.6, 130.2 ± 14.3, and 238.8 ± 3.2 (n = 3); and experiment 3 (small *Daphnia*), below detection limit, 53.1 ± 8.5, 60.8 ± 1.3, 109.2 ± 8.1, and 228.0 ± 2.3 (n = 3). Measured concentrations for the externally supplied cadmium quality assurance samples were always within the certified 95% confidence interval. Recovery (mean ± SD) of cadmium from 14 spiked water samples was 103 ± 1.6% (range 100–106%). The relative standard deviation from analysis of triplicate water samples for cadmium averaged 2.4% (range 0.0–6.0%).

Results

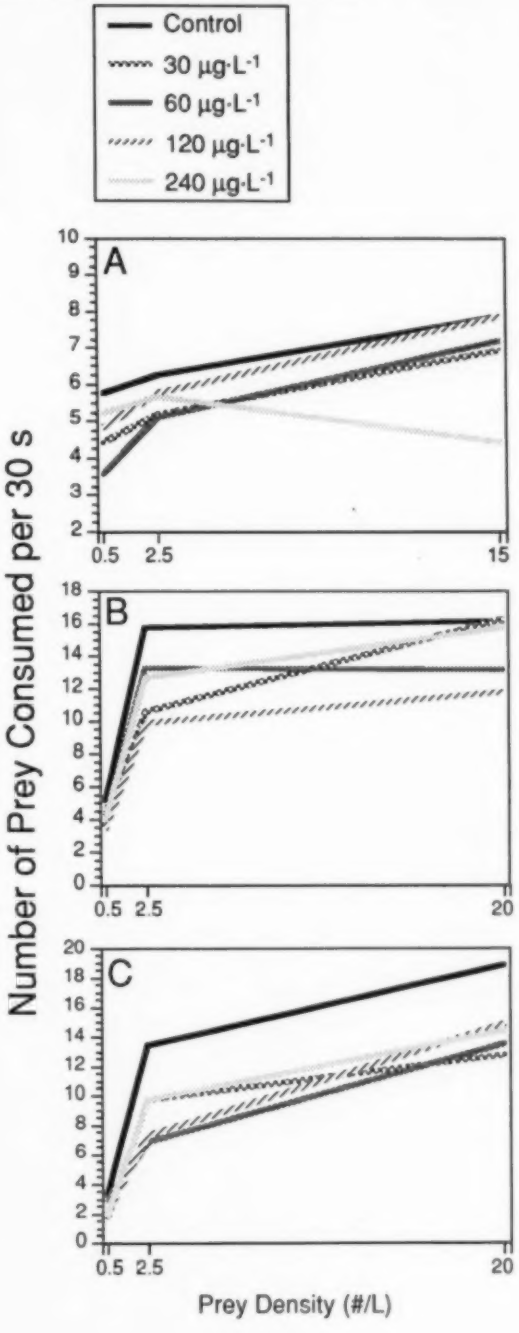
Behavioral effects

Among the five foraging parameters measured, only prey attack and capture rates were significantly ($P \leq 0.05$) affected by cadmium exposure. Because cadmium did not significantly affect prey capture efficiency in any experiment, results from analyzing attack and capture data were essentially the same. Thus, for simplicity and brevity, we only report and discuss cadmium effects on prey attack rates, with the understanding that prey capture rates are affected in the same manner.

The number of prey attacked per 30 s increased significantly with increasing prey density ($P \leq 0.001$) and time ($P \leq 0.006$) in all three experiments. No significant cadmium–density interaction was found in these experiments; however, a significant ($P \leq 0.031$) cadmium–time interaction was determined for all three experiments. With the exception of the 240 μg $Cd \cdot L^{-1}$ treatment, bluegill foraging on large *Daphnia* increased their consumption of prey almost linearly as prey density increased (Fig. 1A). Consumption rates (number eaten/30 s) of bluegill foraging on medium *Daphnia* generally followed a type I functional response (Fig. 1B), whereas consumption rates of bluegill foraging on small *Daphnia* more closely approximated a type II functional response (see Colinvaux 1986) (Fig. 1C).

When data were averaged over prey density, the mean number of prey attacked per 30 s by control fish increased between time periods 1 and 2 in all experiments, whereas attack rates of treated fish remained constant or declined (Fig. 2). Additionally, cadmium effects on bluegill prey attack rates varied substantially with prey size and duration of exposure (Fig. 2). When large *Daphnia* were used as prey, cadmium had no significant effect on the net changes in prey attack rates of bluegill over the course of the 28-d experiment. When bluegill were foraging on medium *Daphnia*, net changes in prey attack rates over this 27-d experiment

Fig. 1. Cadmium effects on the functional response of juvenile bluegill foraging on (A) large (2.5 ± 0.3 mm), (B) medium (1.5 ± 0.2 mm), and (C) small (0.85 ± 0.15 mm) *Daphnia*. Mean (n = 52–56) prey consumption rates of control and cadmium-exposed bluegill are shown at various prey densities. Nominal treatment concentrations are given. Measured mean exposure concentrations for each experiment are reported in the text.



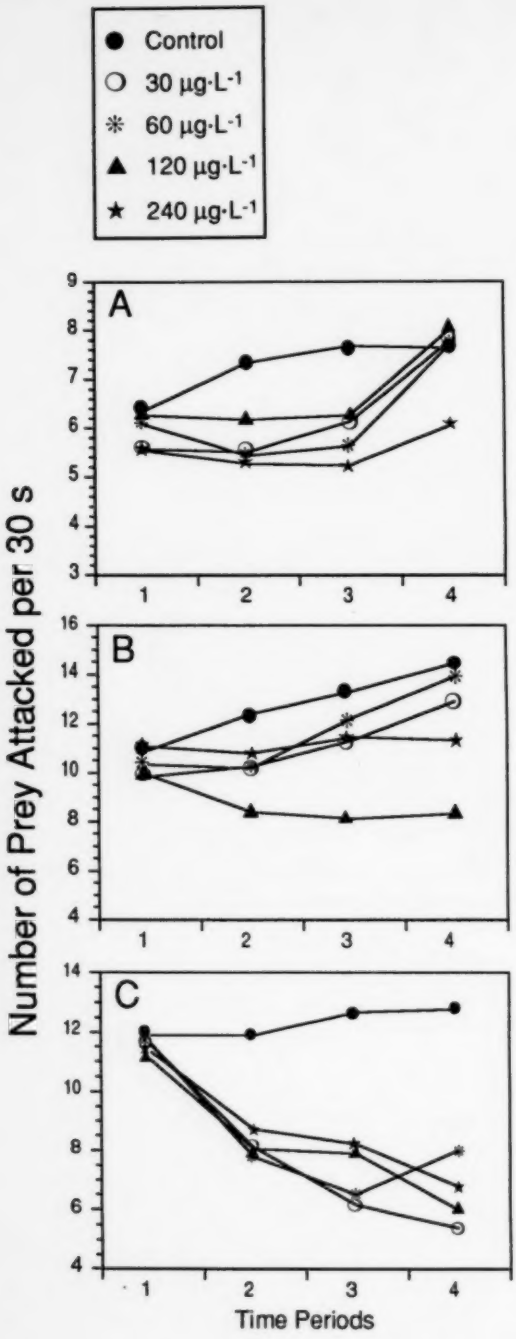


Fig. 2. Cadmium effects on the mean number of (A) large (2.5 ± 0.3 mm), (B) medium (1.5 ± 0.2 mm), and (C) small (0.85 ± 0.15 mm) *Daphnia* attacked by juvenile bluegill during 30-s foraging trials conducted in three separate 4-week experiments. For means in time period 1, n = 48–60, and for means in time periods 2–4, n = 24–36. Nominal treatment concentrations are given. Measured mean exposure concentrations for each experiment are reported in the text. Note scale changes among the three plots.

were significantly reduced ($P \leq 0.050$) in bluegill exposed to 130 and 239 µg Cd·L^{-1}. Finally, when small *Daphnia* were used as prey, net changes in prey attack rates over the 26-d experiment were significantly reduced ($P \leq 0.007$) in bluegill exposed to all cadmium concentrations (Table 1). Hence, when using the attack end point, the LOEC was related to prey size.

Growth effects

In the experiments using medium and small *Daphnia* as prey (where growth effects were assessed), bluegill growth in length and weight was significantly reduced ($P \leq 0.019$) by all cadmium concentrations (Table 2). A no-oberservable-effect concentration (NOEC) could not be determined because there was a significant growth effect at the lowest cadmium concentration established in experiment 2 (37 µg·L^{-1}) and experiment 3 (53 µg·L^{-1}).

Discussion

Cadmium-altered foraging behavior

Changes in prey attack rates of bluegill proved to be the most sensitive behavioral parameter measured for assessing sublethal cadmium stress, with the LOEC being directly related to prey size. Mean prey consumption rates (number eaten/30 s) were initially suppressed in fish exposed to all cadmium concentrations, with recovery to control-level foraging affected by exposure concentration, exposure duration, and prey size. Farmer et al. (1979), Drummond et al. (1973), and Lett et al. (1976) also reported that food consumption by fish was initially reduced by exposure to zinc and copper, but partial or full recovery to control-level feeding occurred within 20 d. Conversely, Collvin (1985) reported that food consumption of perch (*Perca fluviatilis*) exposed to copper for 30 d never returned to control rates after initial suppression.

Physiological and behavioral compensation mechanisms in fish that facilitate recovery of foraging performance during continuous exposure to sublethal metal concentrations remain unclear; however, substantial evidence suggests that metal-binding proteins, such as metallothionein, are likely important (e.g., Kito et al. 1982; Leland and Kuwabara 1985; Hamilton and Mehrle 1986; Fu et al. 1990; Roesijadi 1992). Because of such compensation mechanisms, the sensitivity of behavioral end points may be greatly influenced by the duration of exposure prior to assessment. Thus, in assessing the utility of behavioral assays for hazard assessment, it is important to determine if acute behavioral effects are predictive of chronic effects on growth, survival, or reproduction.

Cadmium effects on the normal increase in prey attack and consumption rates over the course of these experiments

were not only influenced by cadmium concentration, but also by prey size. When large *Daphnia* were used as prey, net changes in prey attack rates over time did not differ significantly among treatments. When medium *Daphnia* were used as prey, only bluegill exposed to the two lower cadmium concentrations recovered from initially suppressed prey attack rates. Finally, when bluegill were foraging on small *Daphnia*, no recovery to control-level foraging was observed for any of the cadmium-treated fish. Cadmium clearly had a greater effect on fish foraging on small than on large prey. If reductions in the number of prey attacked were primarily due to increased prey handling time, then the opposite should have occurred, with changes in prey attack rates being directly related to prey size, not inversely so. Furthermore, cadmium had no significant effect on prey handling time as measured during these experiments. Thus, observations and data from these experiments suggest that exposure to sublethal cadmium concentrations does not affect handling time when feeding on daphnids.

In contrast, Sandheinrich and Atchison (1989) reported that copper significantly increased the handling time of bluegill feeding on large *Daphnia magna* (2.4 mm) and larger invertebrate prey. Increased handling time, in part, resulted from repetitive spitting and recapturing of prey. Zebrafish (*Brachydanio rerio*) exposed to zinc (Cairns and Loos 1967) and lead (Nyman 1981) and flagfish (*Jordanella floridae*) exposed to alkyl benzene sulphonate detergent (ABS) (Foster et al. 1966) also repetitively seized and ejected their prey while foraging. Repetitive spitting and recapturing of prey was not observed in bluegill exposed to cadmium in this study.

Data from other experiments conducted in our laboratory (M.D. Bryan, unpublished data) suggest that sublethal cadmium exposure affects bluegill search strategy by disrupting the normal pause–run pattern of the saltatory search strategy (for descriptions of saltatory search strategies see Janssen 1982; Evans and O'Brien 1988; O'Brien et al. 1986, 1989; Ehlinger 1989, 1990). Degradation of a refined search strategy may reduce food intake by reducing prey encounter rates (Ehlinger 1989, 1990). We feel that reduced prey encounter rates coupled with depressed motivation to feed largely accounted for the reduced prey attack and consumption rates observed in cadmium-exposed fish in this study. Although we attempted to measure motivation to feed in this study, we do not feel that we did so successfully; therefore, depressed feeding motivation in fish exposed to cadmium may indeed contribute to reduced prey attack rates.

Capture efficiency of *Daphnia* was not affected by cadmium exposure, presumably owing to the ease with which bluegill capture and ingest these nonevasive, easily handled prey (Eggers 1977; Drenner et al. 1978; Vinyard 1980; Ehlinger 1989). Studies reporting toxicant-altered capture efficiency (Finger et al. 1985; Mathers et al. 1985; Sandheinrich and Atchison 1989) involved larger and (or) more evasive prey than we used. The sensitivity of the capture-efficiency end point in toxicity testing may be related to the ratio of predator to prey size (Sandheinrich and Atchison 1989) as well as prey evasiveness to attack.

Although we did not observe significant reductions in prey capture efficiency in cadmium-exposed bluegill, the largest deviations in capture efficiency from control levels occurred when using small prey, not when using large prey as one might predict. We hypothesize that the rapidly developing visual acuity of juvenile bluegill (Breck and Gitter 1983; Li et al. 1985; Walton et al. 1992) may be affected by cadmium exposure either directly, or indirectly via cadmium effects on growth. Dial (1978) and Fox and Sillman (1979) have demonstrated adverse effects of heavy metals on fish eye development and rod receptor function, respectively. The visual resolution of juvenile bluegill increases rapidly as fish length increases (Li et al. 1985); thus, cadmium-reduced growth could also impede the development of visual acuity. Cadmium-altered visual acuity

Table 1. Effects of sublethal cadmium exposure on the net change in bluegill prey attack rates (number/30 s) for increasing durations of exposure (test initiation through time period 2, 3, and 4) in three 26- to 28-d experiments with daphnid prey.

	Time period		
	1–2[a]	1–3[b]	1–4[c]
Experiment 1 (large *Daphnia*)			
Main effect			
Cadmium	0.747	0.036	0.801
Contrasts[d]			
0 vs. 26.8 $\mu g \cdot L^{-1}$	0.717	0.104	0.669
0 vs. 61.9 $\mu g \cdot L^{-1}$	0.261	0.006	0.686
0 vs. 121.1 $\mu g \cdot L^{-1}$	0.696	0.029	0.770
0 vs. 242.6 $\mu g \cdot L^{-1}$	0.887	0.018	0.494
Experiment 2 (medium-sized *Daphnia*)			
Main effect			
Cadmium	0.293	0.223	0.036
Contrasts[d]			
0 vs. 37.3 $\mu g \cdot L^{-1}$	0.335	0.490	0.688
0 vs. 64.4 $\mu g \cdot L^{-1}$	0.168	0.673	0.794
0 vs. 130.2 $\mu g \cdot L^{-1}$	0.081	0.054	0.012
0 vs. 238.8 $\mu g \cdot L^{-1}$	0.472	0.332	0.050
Experiment 3 (small *Daphnia*)			
Main effect			
Cadmium	0.154	0.007	0.008
Contrasts[d]			
0 vs. 53.1 $\mu g \cdot L^{-1}$	0.043	0.001	0.001
0 vs. 60.8 $\mu g \cdot L^{-1}$	0.037	0.001	0.007
0 vs. 109.2 $\mu g \cdot L^{-1}$	0.031	0.003	0.002
0 vs. 228.0 $\mu g \cdot L^{-1}$	0.048	0.003	0.003

Note: Analysis of variance tests were performed on the slopes of lines generated by regressing the mean number of attacks/30 s against time for each fish. Numbers reported are probabilities of a greater F value with 4,5 df for the main effect of cadmium and 1,5 df for each orthogonal contrast.

[a]Maximum exposure of 4 d.

[b]Maximum exposure of 15 d.

[c]Maximum exposure of 22 d.

[d]0 values indicate control treatments (no added cadmium).

Table 2. Mean increase in total length and wet weight of juvenile bluegill from two experiments involving chronic exposure to cadmium while feeding on different sized daphnid prey.

Cadmium concentration ($\mu g \cdot L^{-1}$)	Total length (mm)			Weight (mg)		
	$\bar{x}$	SE	$P > F^a$	$\bar{x}$	SE	$P > F^a$
Experiment 2 (medium-sized *Daphnia*)						
0[b]	11.7	0.6	—	0.627	0.044	—
37.3	10.6	0.4	0.004	0.546	0.029	0.019
64.4	10.5	0.4	0.003	0.539	0.037	0.014
130.2	8.7	0.5	<0.001	0.431	0.029	<0.001
238.8	8.2	0.3	<0.001	0.397	0.030	<0.001
Experiment 3 (small *Daphnia*)						
0[b]	9.9	0.8	—	0.502	0.061	—
53.1	8.0	0.5	0.008	0.360	0.032	0.002
60.8	7.2	0.4	0.001	0.296	0.024	<0.001
109.2	7.3	0.4	0.002	0.322	0.029	<0.001
228.0	7.0	0.3	<0.001	0.298	0.024	<0.001

Note: Statistical results are from orthogonal contrasts using analysis of variance procedures. Bluegill were fed a combination of *Daphnia* and frozen brine shrimp (*Artemia salina*). For all means, $n = 12$.

[a]Probability of a greater F value for treatment means compared with control means (F tests with 1,5 df).

[b]Control treatment (no added cadmium).

could have a greater effect on prey capture efficiency in bluegill feeding on small than large prey because small prey are inherently more difficult to locate and track (Confer and Blades 1975; Breck and Gitter 1983). Toxicant-induced reductions in visual acuity would, therefore, result in more misdirected attacks at small than at large prey. The effect of cadmium on developing visual acuity in young fishes has not been studied. Because visual acuity and reactive distance to prey increase rapidly as young fish grow (Breck and Gitter 1983; Li et al. 1985; Blaxter 1986), and because great visual resolution is essential for efficient foraging and growth in young planktivores such as bluegill, this area deserves attention in future toxicological foraging behavior studies.

Cadmium-altered growth

Growth was a more sensitive end point than were feeding behaviors in experiment 2, and produced an equivalent LOEC with that of the attack end point in experiment 3. This suggests that reduced food intake was not the only factor contributing to reduced growth rates in bluegill exposed to cadmium. Elevated daily activity levels, higher resting metabolic rates, and (or) less efficient food assimilation may also have contributed to the cadmium-altered growth rates observed.

Cadmium-treated bluegill in this study occasionally became hyperactive when approached in their treatment tanks or when captured for transfer to a foraging tank. Bouts of hyperactivity were typically short and normally followed by periods of reduced activity or inactivity, even if fish were presented with a foraging opportunity. Ellgaard et al. (1978) also reported hyperactivity in bluegill exposed to 250 $\mu g\ Cd \cdot L^{-1}$. Hyperactivity is characterized by greatly accelerated locomotor activity, overreaction to stimuli, and increased ventilatory activity and is diagnostic of xenobiotics that cause metabolic dysfunction (Drummond and Russom 1990). Cadmium-altered activity levels may directly or indirectly affect feeding motivation and prey search performance. In addition, recent experiments conducted by Atchison et al. (1993) demonstrated that the same cadmium concentrations used in this study significantly increased the basal metabolism of juvenile bluegill, as measured by oxygen consumption rates. Energy used for increased basal or active metabolism is not available for growth (Brett 1979; Collvin 1985).

In addition to changes in metabolism, cadmium-altered food assimilation efficiency may also have contributed to reduced growth in exposed bluegill. For example, Borgmann and Ralph (1986) reported a 50% reduction in food assimilation efficiency and altered growth in white sucker (*Catostomus commersoni*) larvae exposed to 150 $\mu g\ Cd \cdot L^{-1}$. Copper has also been shown to reduce food assimilation efficiency in rainbow trout (*Oncorhynchus mykiss*) (Waiwood and Beamish 1978) and yellow perch (*Perca fluviatilis*) (Collvin 1985).

Although growth was the most sensitive indicator of chronic cadmium toxicity to juvenile bluegill in this study, physiological and behavioral effects of cadmium are not discrete or independent. Physiological effects that lead to observed hyperactivity may adversely affect foraging behavior, subsequently reducing prey consumption and exacerbating the effects that increased metabolism alone would have on growth. In this study, cadmium-altered foraging behaviors contributed to reduced growth. In fact, the manner

in which fish were fed throughout these experiments may, in part, explain why the LOEC for growth derived from this study was lower than the LOEC of 80 μg $Cd \cdot L^{-1}$ reported by Eaton (1974) for bluegill chronically exposed to cadmium in hard water (200 $mg \cdot L^{-1}$ as $CaCO_3$). Fish in this study had to pursue and capture live prey or feed efficiently on frozen brine shrimp during short daily foraging periods, meant to simulate the foraging time – predation risk trade-off that juvenile bluegill face in the wild (Werner and Hall 1988). Reduced food consumption, episodes of hyperactivity, and elevated basal metabolism probably all contributed to the reduced growth rates measured in exposed bluegill in this study.

Choosing predator–prey species combinations that are ecologically realistic for laboratory study is, therefore, critical for accurate assessment and prediction of a toxicant's effects on foraging and growth in the field. Standardized growth tests that use artificial prey (e.g., trout pellets) remove important behaviors from the foraging process that are the basis and means of food acquisition in the wild. Growth assays conducted in this manner have been found to be less sensitive for determining LOECs than tests assessing survival and reproduction in more than 70% of the studies reviewed (Woltering 1984). The insensitivity of such growth assays may largely be due to the manner in which they were conducted.

Acknowledgements

Funding for this project was provided by the Environmental Protection Agency (grant No. R-815921-01-0) and the Iowa State University Agriculture and Home Economics Experiment Station. We thank Dr. M. Kaiser and Dr. D. Cox for statistical consultation, and B. Knights, T. Rosenburg, D. Henshaw, J. Grady, and J. Gammack for their laboratory and field assistance.

References

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1989. Standard methods for the examination of water and wastewater. 17th ed. American Public Health Association, Washington, D.C.

American Society for Testing and Materials. 1990. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. ASTM E729-88. American Society for Testing and Materials, Philadelphia, Pa.

Atchison, G.J., M.G. Henry, and M.B. Sandheinrich. 1987. Effects of metals on fish behavior: a review. Environ. Biol. Fishes, 18: 11–25.

Atchison, G.J., M.D. Bryan, and M.B. Sandheinrich. 1993. Fish behavior tests, ecological foraging models and bioenergetics models for risk assessment. U.S. Environ. Prot. Agency Res. Dev. Rep. EPA. Grant No. R-815921-01-0.

Beitinger, T.L. 1990. Behavioral reactions for the assessment of stress in fishes. J. Gt. Lakes Res. 16: 495–528.

Blaxter, J.H.S. 1986. Development of sense organs and behavior of teleost larvae with special reference to feeding and predator avoidance. Trans. Am. Fish. Soc. 115: 98–114.

Borgmann, U., and K.M. Ralph. 1986. Effects of cadmium, 2,4-dichlorophenol, and pentachlorophenol on feeding, growth, and particle-size-conversion efficiency of white sucker larvae and young common shiners. Arch. Environ. Contam. Toxicol. 15: 473–480.

Breck, J.E., and M.J. Gitter. 1983. Effect of fish size on the reactive distance of bluegill (*Lepomis macrochirus*) sunfish. Can. J. Fish. Aquat. Sci. 40: 162–167.

Brett, J.R. 1979. Environmental factors and growth. Chap. 10. *In* Fish physiology. Vol. 8. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York.

Cairns, J., Jr., and J.J. Loos. 1967. Changed feeding rate of *Brachydanio rerio* (Hamilton-Buchanan) resulting from exposure to sublethal concentrations of zinc, potassium dichromate, and alkyl benzene sulfonate detergent. Proc. Pa. Acad. Sci. 40: 47–52.

Colinvaux, P.A. 1986. Ecology. John Wiley & Sons, Inc., New York.

Collvin, L. 1985. The effects of copper on growth, food consumption and food conversion of perch *Perca fluviatilis* L. offered maximal food rations. Aquat. Toxicol. 6: 105–113.

Confer, J.L., and P.I. Blades. 1975. Reaction distance to zooplankton by *Lepomis gibbosus*. Verh. Int. Ver. Theor. Angew. Limnol. 19: 2493–2497.

Dial, N.A. 1978. Some effects of methylmercury on development of the eye in medaka fish. Growth, 42: 309–318.

Drenner, R.W., J.R. Strickler, and W.J. O'Brien. 1978. Capture probability: the role of zooplankter escape in the selective feeding of planktivorous fish. J. Fish. Res. Board Can. 35: 1370–1373.

Drummond, R.A., and C.L. Russom. 1990. Behavioral toxicity syndromes: a promising tool of assessing toxicity mechanisms in juvenile fathead minnows. Environ. Toxicol. Chem. 9: 37–46.

Drummond, R.A., W.A. Spoor, and G.F. Olson. 1973. Some short-term indicators of sublethal effects of copper on brook trout, *Salvelinus fontinalis*. J. Fish. Res. Board Can. 30: 698–701.

Eaton, J.G. 1974. Chronic cadmium toxicity to bluegill (*Lepomis macrochirus* Rafinesque). Trans. Am. Fish. Soc. 103: 729–735.

Eggers, D.M. 1977. The nature of prey selection by planktivorous fish. Ecology, 58: 46–59.

Ehlinger, T.J. 1989. Learning and individual variation in bluegill foraging: habitat-specific techniques. Anim. Behav. 38: 643–658.

Ehlinger, T.J. 1990. Habitat choice and phenotypic-limited feeding efficiency in bluegill: individual differences and trophic polymorphism. Ecology, 71: 886–896.

Eisler, R. 1985. Cadmium hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Wildl. Serv. Biol. Rep. No. 85(1.2).

Ellgaard, E.G., J.E. Tusa, and A.A. Malizia, Jr. 1978. Locomotor activity of the bluegill *Lepomis macrochirus*: hyperactivity induced by sublethal concentrations of cadmium, chromium and zinc. J. Fish Biol. 12: 19–23.

Evans, B.I., and W.J. O'Brien. 1988. A reevaluation of the search cycle of planktivorous Arctic grayling, *Thymallus arcticus*. Can. J. Fish. Aquat. Sci. 45: 187–192.

Everest, F.H., and E.H. Edmundson. 1967. Cold branding for field use in marking juvenile salmonids. Prog. Fish-Cult. 29: 175–176.

Farmer, G.J., D. Ashfield, and H.S. Samant. 1979. Effects of zinc on juvenile Atlantic salmon *Salmo salar*: acute toxicity, food intake, growth and bioaccumulation. Environ. Pollut. 19: 103–117.

Finger, S.E., E.E. Little, M.G. Henry, J.F. Fairchild, and T.P. Boyle. 1985. Comparison of laboratory and field assessment of fluorine. Part I. Effects of fluorine on the survival, growth, reproduction, and behavior of aquatic organisms in

laboratory tests. *In* Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems. *Edited by* T.P. Boyle. ASTM Spec. Tech. Publ. No. 865. pp. 120–133.

Foster, N.R., A. Scheier, and J. Cairns, Jr. 1966. Effects of ABS on feeding behavior of flagfish, *Jordanella floridae*. Trans. Am. Fish. Soc. 95: 109–110.

Fox, D.A., and A.J. Sillman. 1979. Heavy metals affect rod, but not cone, photoreceptors. Science (Washington, D.C.), 206: 78–80.

Fu, H., O.M. Steinebach, C.J.A. Van den Hamer, P.H.M. Balm, and R.A.C. Lock. 1990. Involvement of cortisol and metallothionein-like proteins in the physiological responses of tilapia (*Oreochromis mossambicus*) to sublethal cadmium stress. Aquat. Toxicol. 16: 257–270.

Gardiner, J. 1974. The chemistry of cadmium in natural water. II. The adsorption of cadmium on river muds and naturally occurring solids. Water Res. 8: 157–164.

Hamilton, S.J., and P.M. Mehrle. 1986. Metallothionein in fish: review of its importance in assessing stress from metal contamination. Trans. Am. Fish. Soc. 115: 596–609.

Hart, P.J.B. 1986. Foraging in teleost fishes. *In* The behavior of teleost fishes. *Edited by* T.J. Pitcher. The Johns Hopkins University Press, Baltimore, Md. pp. 211–235.

Henry, M.G., and G.J. Atchison. 1991. Metal effects on fish behavior. Advances in determining the ecological significance of responses. *In* Ecotoxicology of metals: current concepts and applications. *Edited by* M.C. Newman and A.W. McIntosh. Lewis Publishers, Inc., Chelsea, Mich. pp. 131–143.

Janssen, J. 1982. Comparison of searching behavior for zooplankton in the obligate planktivore, blueback herring (*Alosa aestivalis*) and a facultative planktivore, bluegill (*Lepomis macrochirus*). Can. J. Fish. Aquat. Sci. 39: 1649–1654.

Kay, S.H. 1985. Cadmium in aquatic food webs. Residue Rev. 96: 13–43.

Kito, H., T. Tazawa, Y. Ose, T. Sato, and T. Ishikawa. 1982. Protection by metallothionein against cadmium toxicity. Comp. Biochem. Physiol. C, 37: 135–139.

Leland, H.V., and J.S. Kuwabara. 1985. Trace metals. *In* Fundamentals of aquatic toxicology: methods and applications. *Edited by* G.M. Rand and S.R. Petrocelli. Hemisphere Publishing Corp., New York. pp. 374–415.

Lett, P.F., G.J. Farmer, and F.W.H. Beamish. 1976. Effects of copper on some aspects of the bioenergetics of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 33: 1335–1342.

Levin, S.A., K.D. Kimball, W.H. McDowell, and S.F. Kimball. 1984. New perspectives in ecotoxicology. Environ. Manage. 8: 375–442.

Li, K.T., J.K. Wetterer, and N.G. Hairston, Jr. 1985. Fish size, visual resolution, and prey selectivity. Ecology, 66: 1729–1735.

Little, E.E. 1990. Behavioral toxicology: stimulating challenges for a growing discipline. Environ. Toxicol. Chem. 9: 1–2.

Little, E.E., B.A. Flerov, and N.N. Ruzhinskaya. 1985. Behavioral approaches in aquatic toxicity investigations: a review. *In* Toxic substances in the environment: an international aspect. *Edited by* P.M. Mehrle, R.K. Gray, and R.L. Kendall. Water Quality Section, American Fisheries Society, Bethesda, Md. pp. 351–376.

Maki, A.W. 1979. An analysis of decision criteria in environmental hazard evaluation programs. *In* Analyzing the hazard evaluation process. *Edited by* K.L. Dickson, A.W. Maki, and J. Cairns, Jr. Water Quality Section, American Fisheries Society, Bethesda, Md. pp. 83–100.

Mathers, R.A., J.A. Brown, and P.H. Johansen. 1985. The growth and feeding behavior responses of largemouth bass (*Micropterus salmoides*) exposed to PCP. Aquat. Toxicol. 6: 157–164.

Nriagu, J.O., and J.B. Sprague. (*Editors*). 1987. Cadmium in the aquatic environment. Vol. 19. John Wiley and Sons, New York.

Nyman, H.G. 1981. Sublethal effects of lead (Pb) on size selective predation by fish: applications on the ecosystem level. Verh. Int. Ver. Theor. Angew. Limnol. 21: 1126–1130.

O'Brien, W.J., B.I. Evans, and G.L. Howick. 1986. A new view of the predation cycle of a planktivorous fish, white crappie (*Pomoxis annularis*). Can. J. Fish. Aquat. Sci. 43: 1894–1899.

O'Brien, W.J., B.I. Evans, and H.I. Browman. 1989. Flexible search tactics and efficient foraging in saltatory searching animals. Oecologia, 80: 100–110.

Rand, G.M. 1985. Behavior. *In* Fundamentals of aquatic toxicology: methods and applications. *Edited by* G.M. Rand and S.R. Petrocelli. Hemisphere Publishing Corp., New York. pp. 221–263.

Roesijadi, G. 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat. Toxicol. 22: 81–114.

Sandheinrich, M.B., and G.J. Atchison. 1989. Sublethal copper effects on bluegill, *Lepomis macrochirus*, foraging behavior. Can. J. Fish. Aquat. Sci. 46: 1977–1985.

Sandheinrich, M.B., and G.J. Atchison. 1990. Sublethal toxicant effects on fish foraging behavior: empirical vs. mechanistic approaches. Environ. Toxicol. Chem. 9: 107–119.

SAS Institute Inc. 1985. SAS user's guide: statistics. Version 5 edition. SAS Institute Inc., Cary, N.C.

Scherer, E. 1992. Behavioral responses as indicators of environmental alterations: approaches, results, developments. J. Appl. Ichthyol. 8: 122–131.

Taylor, J.K. 1987. Quality assurance of chemical measurements. Lewis Publishers, Inc., Chelsea, Mich.

U.S. Environmental Protection Agency. 1985. Ambient water quality criteria for cadmium: 1984. EPA 440/5-84-032.

Vinyard, G.L. 1980. Differential prey vulnerability and predator selectivity: effects of evasive prey on bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) predation. Can. J. Fish. Aquat. Sci. 37: 2294–2299.

Waiwood, K.G., and F.W.H. Beamish. 1978. The effect of copper, hardness and pH on growth of rainbow trout (*Salmo gairdneri*). J. Fish Biol. 13: 591–598.

Walton, W.E., N.G. Hairston, Jr., and J.K. Wetterer. 1992. Growth-related constraints on diet selection by sunfish. Ecology, 73: 429–437.

Werner, E.E., and D.J. Hall. 1988. Ontogenetic habitat shifts in bluegill: the foraging rate-predation risk trade-off. Ecology, 69: 1352–1366.

Woltering, D.M. 1984. The growth response in fish chronic and early life stage toxicity tests: a critical review. Aquat. Toxicol. 5: 1–21.

Effect of predation by Sacramento squawfish (*Ptychocheilus grandis*) on habitat choice of California roach (*Lavinia symmetricus*) and rainbow trout (*Oncorhynchus mykiss*) in artificial streams

Larry R. Brown and Anne M. Brasher

Abstract: We studied the effects of predatory Sacramento squawfish (*Ptychocheilus grandis*) on habitat choice of juvenile California roach (*Lavinia symmetricus*), adult roach, and juvenile rainbow trout (*Oncorhynchus mykiss*) in artificial streams. In single-prey trials, the proportion of fish found in pool habitat declined in the presence of squawfish for juvenile roach (from 0.55 to 0.00), adult roach (from 0.88 to 0.13), and juvenile rainbow trout (0.70 to 0.15). The presence of squawfish did not affect the use of riffle and edge habitats. Of the fish found in shallow water, the proportion found in edge habitat declined from juvenile roach (0.95) to adult roach (0.80) to juvenile rainbow trout (0.23). We also conducted experiments designed to simulate invasion of an area by squawfish, in which adult roach and juvenile rainbow trout were tested together in the presence and absence of squawfish. The proportion of prey in pool habitat in the presence of squawfish was greater in the two-prey trials than in the single-prey trials for both adult roach (0.31 and 0.13, respectively) and juvenile rainbow trout (0.33 and 0.15, respectively). These results support field evidence that squawfish are an important force in determining the spatial structure of native stream fish assemblages.

Résumé : Nous avons étudié l'effet de la présence de la sauvagesse du Sacramento (*Ptychocheilus grandis*), un prédateur, sur le choix d'habitats par les juvéniles et les adultes du gardon de Californie (*Lavinia symmetricus*) ainsi que par les juvéniles de la truite arc-en-ciel (*Oncorhynchus mykiss*) dans des cours d'eau créés artificiellement. Lors d'essais portant sur un seul type de proie, l'abondance de poissons trouvés dans les trous d'eau en présence de la sauvagesse, diminuait dans l'ordre suivant : juvéniles du gardon (de 0,55 à 0,00), adultes du gardon (de 0,88 à 0,13), juvéniles de la truite (de 0,70 à 0,15). La présence de la sauvagesse n'avait aucun effet sur l'utilisation des habitats des seuils et des écotones. Des poissons trouvés en eaux peu profondes, l'abondance de ceux trouvés dans les habitats des écotones diminuait dans l'ordre suivant : juvéniles du gardon (0,95), adultes du gardon (0,80), juvéniles de la truite (0,23). Nous avons aussi procédé à des expériences dont l'objectif était de simuler l'invasion d'un secteur par la sauvagesse en plaçant dans ce secteur des adultes du gardon et des juvéniles de la truite en présence ou non de la sauvagesse. Tant chez les adultes du gardon (0,31 et 0,13, respectivement) que chez les juvéniles de la truite (0,33 et 0,15, respectivement), il y avait davantage de proies dans les trous d'eau lorsqu'on y introduisait les deux espèces que lorsqu'on n'en introduisait qu'une, en présence de la sauvagesse. Ces résultats confirment les observations sur le terrain indiquant que la sauvagesse contribue fortement à déterminer la répartition dans l'espace des communautés de poissons indigènes des cours d'eau.

[Traduit par la Rédaction]

Received April 7, 1994. Accepted March 2, 1995.
J12333

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Introduction

Predation is a major determinant of the ecological structure of stream fish assemblages (Fraser and Emmons 1984; Power 1987; Schlosser 1987; Brown and Moyle 1991; Harvey 1991; Harvey and Stewart 1991; Angermeier 1992). In particular, the presence of predatory fishes can influence patterns of habitat (pool, riffle, run) and microhabitat (position within a habitat) use among potential prey (Power et al. 1985; Schlosser 1987; Brown and Moyle 1991; Harvey 1991). The majority of predation studies in the United States have been conducted in eastern and central region streams and have emphasized the effects of predatory centrarchids (Power et al. 1985; Schlosser 1987; Harvey 1991; Angermeier 1992) and cyprinids (Cerri and Fraser 1983; Fraser and Emmons 1984; Schlosser 1988; Schlosser and Ebel 1989) on native stream fish assemblages. In western North America, predation studies have tended to focus on the effects of predators, including squawfish (*Ptychocheilus* spp.), on commercially important salmonids (Brown and Moyle 1981; Rieman et al. 1991). Increasing interest in conservation of native aquatic ecosystems, including fish assemblages that do not include commercially important species, makes the need to understand interrelationships among native species, including predator–prey relationships, more urgent. In addition, because the intentional and accidental introduction of exotic species, including predators, into native aquatic communities is common and the results are often unpredictable (Moyle 1986; Moyle et al. 1986), the effects of such introductions need to be understood if conservation attempts are to succeed.

In much of western North America, the dominant native stream-dwelling piscivores are salmonids or squawfish. Squawfish predation has been the focus of previous studies because of their ability to consume large numbers of juvenile salmonids, especially in systems subject to human alterations (Brown and Moyle 1981; Rieman et al. 1991; Vigg et al. 1991). Recent studies of streams supporting populations of Sacramento squawfish, *Ptychocheilus grandis*, suggest that these fish may play a major role in structuring many California stream fish communities (Moyle and Baltz 1985; Brown and Moyle 1991; Baltz and Moyle 1993). In particular, Brown and Moyle (1991) documented habitat and microhabitat shifts by resident fishes in the Eel River, California, in response to invasion by introduced Sacramento squawfish (squawfish for the remainder of the paper). Squawfish were introduced into the Eel River in about 1980; allowing Brown and Moyle (1991) to compare habitat use of resident fishes in similar areas with and without squawfish (South Fork Eel River) and in the same area (Van Duzen River) before and after squawfish invasion. However, the responses of some species and size-classes varied between the two field sites. For example, juvenile rainbow trout were completely absent from pool habitat in the presence of squawfish in the South Fork Eel River but juvenile rainbow trout were still present in squawfish-inhabited pools in the Van Duzen River, though the proportion of the population observed in pools was lower than when squawfish were absent. Brown and Moyle (1991) suggested that the observed differences in prey responses may have been due to differences in availability of refuges from predation. The implication is that the prey are able to assess predation risk and adjust their habitat utilization accordingly.

The purpose of this study was to examine the effect of predation by squawfish on habitat choice by two commonly encountered prey species under controlled experimental conditions. We examined habitat choice of California roach (*Lavinia symmetricus*) and rainbow trout (*Oncorhynchus mykiss*) in artificial streams and determined if changes occurred in the presence of predatory squawfish (≥200 mm SL (standard length)). These two species commonly occur with squawfish in streams throughout California (Moyle 1976). We also examined habitat choice of adult California roach (roach for the remainder of the paper) and juvenile rainbow trout in two-prey trials compared with single-prey trials. Because our intention was to assess possible interactions of the two prey during invasion of their habitat by squawfish, we simulated crowding by setting the total density of the two-prey trials as the sum of the single-prey trial densities (i.e., density of two-prey trial = density of adult roach in single-prey trial + density of juvenile rainbow trout in single-prey trial). We addressed the following questions. 1. Does the habitat choice of juvenile roach, adult roach, or juvenile rainbow trout change in the presence of predatory squawfish? 2. Does the habitat choice of juvenile roach, adult roach, or juvenile rainbow trout vary among species or size-class and does the level of response to the presence of squawfish vary among prey? 3. When tested in mixed-prey (high density) trials, do adult roach and juvenile rainbow trout affect the habitat choice or the response to the presence of squawfish of the other species compared with single-prey (low density) trials? The answers to these questions will provide information on predator–prey relationships among commonly co-occurring species that will aid in understanding processes structuring native stream fish assemblages.

Materials and methods

Fish

Squawfish used in the experiments were captured from the main-stem Eel River on 8 May 1990 using a boat electroshocker. Squawfish ranged in size from 270 to 320 mm SL. Squawfish larger than 200 mm SL feed primarily on fish, taking other prey such as frogs and crayfish when available (Brown 1991; L.R. Brown, unpublished data). A total of 12 squawfish were maintained as a predator pool in a 3.3 m diameter fiberglass holding tank. Four squawfish were selected from this pool at the beginning of each of six sets of experimental runs. Sets ranged in size from two to three experimental runs and were separated by periods of time ranging from 5 to 38 days depending on weather and availability of personnel. Squawfish were returned to the holding tank after completion of each set of experimental runs. Squawfish were not measured at the end of each set because of the relatively uniform size of the fish and the possibility of injuring them. During the interim periods, squawfish were supplied with live fish as food. Food species included roach, juvenile Sacramento suckers (*Catostomus occidentalis*), and juvenile squawfish. Roach used in the experiments were captured with seines

from the South Fork Eel River and Sprowel Creek, a tributary. Rainbow trout were captured with a backpack electroshocker from Sprowel Creek. All experimental prey fish were held overnight in a holding tank (3 m long × 0.5 m wide or 3.3 m diameter) before being used in a trial. Prey fish were only used once in experimental trials.

Artificial streams

The experimental streams consisted of four rectangular, fiberglass tanks located downslope from a salmonid rearing facility on Little Sprowel Creek, a second-order tributary to Sprowel Creek, which joins the South Fork Eel River near Garberville, California (40°05′N, 123°50′W). The tanks were fitted with a plywood insert to create two parallel shallow water compartments (1.8 m long × 0.5 m wide) at the upstream end of the tank and a single deep water compartment (1.75 m long × 1.1 m wide) at the downstream end (Fig. 1). Water was supplied to the tanks from the rearing facility by gravity flow, through 4-cm polyvinyl chloride (PVC) pipe. In each tank a spray bar was directed at the surface of the upstream end of one of the shallow compartments. Water exited through a standpipe in the downstream deep water compartment. Flow was regulated using valves and varied from 0.5 to 0.8 $L \cdot s^{-1}$ in individual tanks. The water was very clear and the bottom of deep water compartments was clearly visible from the surface at all times. Daytime water temperatures varied from 11 to 19°C from 13 May to 25 August 1990, when experiments were conducted.

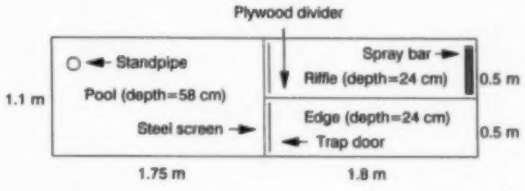
Compartments were configured to simulate three habitat types: riffle, pool, and stream edge. The compartment with the spray bar provided shallow, turbulent conditions meant to simulate riffle habitat. The deep water compartment provided deep, calm conditions representing pool habitat. The remaining compartment presented fish with shallow, calm conditions meant to simulate stream edge habitat. Squawfish were excluded from the shallow water habitats by 2.5-cm mesh steel screen. The compartments in each tank could be isolated simultaneously using plastic drop gates. The substrate in all habitats consisted of gravel approximately 2.5 cm in diameter. The depth (mean ± SD) in the deep water compartments was 58 ± 2 cm and in the shallow compartments it was 24 ± 4 cm. The spray bar created turbulence and eddies so velocities at any point in the system ranged from positive to negative. The spray bar simulated the turbulence of riffle habitat but did not provide unidirectional water velocities. The tanks were fitted with 0.6-cm hardware cloth lids. Shade was provided by a 60 cm wide strip of nylon sunscreen running the length of the center of the tank and fastened to the hardware cloth lid.

Clearly, we could not simulate natural habitat in the Eel River system where stream width often exceeds 30 m and depths can exceed 3 m (L.R. Brown, unpublished data). The stream design provided prey with clear options in habitat choice, while providing sufficient space for the predator to capture prey. Gravel substrate is common in all habitats of the Eel River system during summer flow conditions and is often the dominant substrate available in pools, riffles, and runs.

Experimental design

Single-prey experiments were conducted to determine habitat choice of juvenile roach (33 ± 3 mm SL, mean ± SD), adult roach (53 ± 7 mm SL), and juvenile rainbow trout (39 ± 9 mm SL) in the presence and absence of squawfish. In each experimental run, two tanks were used as squawfish treatments and two tanks acted as controls. We placed two squawfish in the pool habitat compartment of each treatment tank. The high density of squawfish was meant to create an environment for prey where the risk of predation was high. Two squawfish were used to make sure a threat of predation was present in the eventuality that one of the predators died during a trial (no mortality occurred). With the drop gates down, we placed six prey of a single species or size-class in each shallow water compartment of each tank for a total density of 3 prey/m^2. These prey densities were within the range of natural variability for the Eel River drainage (L.R. Brown and P.B. Moyle, unpublished data). We then covered the stream tanks and raised the drop gates, giving the prey access to the pool habitat. Predators could not enter the shallow water habitats because of the steel screens. We left the stream tanks undisturbed, overnight (approximately 24 h) for the two size-classes of roach and for 2 nights (approximately 48 hours) for the rainbow trout. At the end of the trial, the gates were dropped and the number of fish in each habitat type was counted.

Fig. 1. Top view of the artificial streams used in the experiments.



Preliminary data for roach collected in 1989 indicated that the results were not statistically different between trials run for 1 or 2 nights. Data collected during one set of single-prey rainbow trout experiments indicated no significant difference between results after 1 or 2 nights (repeated-measures ANOVA of proportion of fish in pool habitat (see statistical comparisons), all time effects $P > 0.05$) and supported qualitative observations made during 1989 preliminary trials. This result for rainbow trout allowed the comparison of results among species for the single-prey trials and the use of an overnight test period in the two-prey trials.

We conducted two-prey experiments using adult roach (61 ± 6 mm SL) and juvenile rainbow trout (47 ± 9 mm SL) as prey simultaneously. Because the intent of the experiment was also to simulate the effect of fish crowding together in response to the invasion of an area by squawfish, we used 12 fish of each species in the trials for a total of 24 prey fish/tank (6 prey/m^2). We ran these experiments overnight (approximately 24 h).

We assumed that prey fish that were not recovered were eaten because >99% of the fish from the control tanks (no squawfish present) were recovered. Squawfish were able to capture all prey types under the experimental conditions provided (Table 1). Four experimental runs were conducted for each prey type, giving a total of two replicates per stream tank for each predator treatment (presence or absence).

Table 1. Mean number of prey eaten, standard error (SE), minimum, and maximum for single-prey and two-prey trials.

Species	Trial type	Mean	SE	Minimum	Maximum
Adult roach	Single prey (24 h)	3.9	1.1	0	8
Juvenile roach	Single prey (24 h)	2.6	0.5	1	5
Rainbow trout	Single prey (48 h)	3.7	0.8	1	8
Adult roach	Two prey (24 h)	2.6	0.9	0	8
Rainbow trout	Two prey (24 h)	2.1	0.9	0	6

Note: Sample size was 8 for all means.

Statistical comparisons

We used analysis of variance to evaluate the effects of squawfish on two response variables, the proportion of the total number of fish in the artificial stream found in pool habitat and the proportion of the total number of fish using shallow habitat found in edge habitat. For example, if 6 fish were recovered from pool habitat, 4 from edge habitat, and 2 from riffle habitat the appropriate proportions for analysis would be a proportion of 0.50 (6/12) of fish in pool habitat and 0.67 (4/6) of shallow water fish in edge habitat.

Proportions were arcsine transformed before analysis. In trials when no fish were found in shallow water, a mean value calculated from results of the other trials in the appropriate treatment was used. In some cases there was complete use of edge habitat in a treatment. In these cases, one value was adjusted to 0.917 (value for 11 fish in edge habitat of 12 fish found in shallow water). The use of proportions avoided the complicating factor of reductions in the number of prey as a result of predation. We did not compare the predation rates of squawfish on the different prey because we made no attempt to standardize squawfish activity or predation behavior between experimental runs or sets of experimental runs. Also, representing the result of each trial by a single value rather than treating each prey fish as an independent result is statistically conservative.

We compared the results of the single-prey trials among prey using three-way analysis of variance of prey type, presence of squawfish, and stream tank. Separate analyses were conducted for each of the two response variables.

We tested for adult roach – juvenile rainbow trout interactions by comparing the results of single-prey (low density) trials with those of two-prey (high density) trials. We used three-way analysis of variance with prey type, presence of squawfish, and presence of the other species (density) as factors. Separate analyses were conducted for each of the two response variables. For both single-prey and two-prey trials, Bonferroni comparisons were used for a posteriori tests.

Results

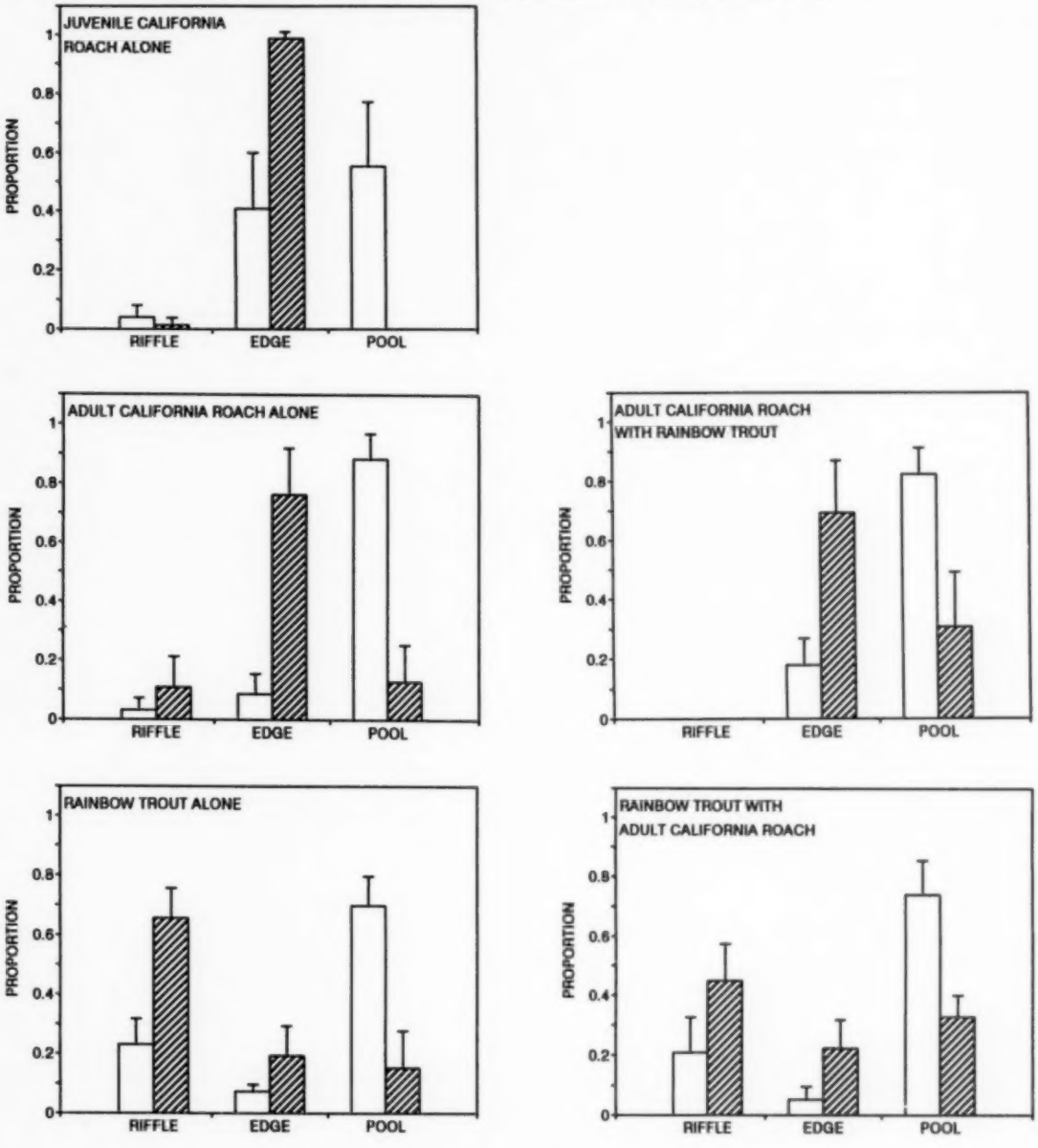
The presence or absence of squawfish had a strong effect on how juvenile roach, adult roach, and rainbow trout used pool habitat in the single-prey trials ($F_{[1,24]} = 129.0$, $P < 0.001$). All prey groups reduced their use of pool habitat in the presence of squawfish (Fig. 2). Use of pool habitat declined from 0.55 ± 0.30 (mean ± SD) ($n = 8$) to 0.00 ± 0.00 ($n = 8$) for juvenile roach, from 0.88 ± 0.12 ($n = 8$) to 0.13 ± 0.18 ($n = 8$) for adult roach, and from 0.70 ± 0.15 ($n = 8$) to 0.15 ± 0.18 ($n = 8$) for juvenile rainbow trout. The three prey groups differed in the proportion of fish using pool habitat, independent of the presence of squawfish ($F_{[2,24]} = 6.7$, $P = 0.005$), with a mean proportion of 0.28 ± 0.35 ($n = 16$) for juvenile roach, 0.51 ± 0.42 ($n = 16$) for adult roach, and 0.43 ± 0.32 ($n = 16$) for juvenile rainbow trout. Bonferroni comparisons indicated that juvenile roach used pool habitat significantly less often than adult roach ($P < 0.001$) and juvenile rainbow trout ($P = 0.001$). The proportions of adult roach and juvenile rainbow trout using the pool habitat were not significantly different.

The proportion of fish using edge habitat of those found in shallow water varied significantly only with prey group ($F_{[2,24]} = 32.6$, $P < 0.001$). The proportion of shallow water fish using edge habitat declined from juvenile roach (0.95 ± 0.10, $n = 15$) to adult roach (0.80 ± 0.31, $n = 13$) to juvenile rainbow trout (0.23 ± 0.16, $n = 16$). Bonferroni comparisons among prey indicated that adult and juvenile roach were similar but both were different from juvenile rainbow trout ($P < 0.001$).

The presence or absence of squawfish strongly affected the proportion of fish using pool habitat in the comparison of two-prey (high density) and single-prey (low density) trials ($F_{[1,56]} = 129.0$, $P < 0.001$). Both adult roach and rainbow trout used pool less often when squawfish were present (Fig. 2). The proportion of fish using pool habitat declined from 0.85 ± 0.12 ($n = 16$) to 0.22 ± 0.24 ($n = 16$) for adult roach and from 0.72 ± 0.15 ($n = 16$) to 0.24 ± 0.17 ($n = 16$) for juvenile rainbow trout. The greater shift out of pool habitat by adult roach was statistically significant (significant interaction between prey group and the presence of squawfish, $F_{[1,56]} = 5.1$, $P = 0.027$). The response of the prey groups to the presence or absence of squawfish was affected by the presence of the other prey (higher density) as indicated by a significant interaction between the two factors ($F_{[1,56]} = 6.5$, $P = 0.014$). Greater proportions of both prey were found in pool habitat in the presence of squawfish in the two-prey trials than in the single-prey trials (Fig. 2). Values increased from 0.13 ± 0.18 ($n = 8$) to 0.31 ± 0.26 ($n = 8$) for adult roach and from 0.15 ± 0.18 ($n = 8$) to 0.33 ± 0.11 ($n = 8$) for juvenile rainbow trout.

The presence or absence of squawfish did not affect the proportion of shallow water fish found in edge habitat in the comparison of two-prey (high density) and single-prey (low density) trials ($P = 0.077$); however, the prey groups

Fig. 2. Habitat choice by adult California roach, juvenile California roach, and juvenile rainbow trout in artificial streams during single-prey trials and by adult California roach and juvenile rainbow trout in two-prey trials in the presence (hatched bars) and absence (open bars) of Sacramento squawfish. Data are given as the mean proportion of fish recovered from a habitat type ± 2 SE. The number of replicates was 8 for each set of bars.



were different ($F_{[1,56]}$ = 131.0, $P < 0.001$). Adult roach were found more often in edge habitat (0.91 ± 0.23, n = 28) than were juvenile rainbow trout (0.27 ± 0.21, n = 32). Both prey used edge habitat to a greater extent in the two-prey (high density) trials than in the single-prey (low density) trials ($F_{[1,56]}$ = 6.8, P = 0.012, Fig. 2). Proportions increased from 0.80 ± 0.31 (n = 13) to 1.00 ± 0.00 (n = 15) for adult roach and from 0.23 ± 0.16 (n = 16) to 0.31 ± 0.26 (n = 16) for juvenile rainbow trout. The presence of adult roach affected the response of juvenile rainbow trout to the presence of squawfish to a greater degree than the presence of juvenile rainbow trout affected the response of adult roach (three-way interaction, $F_{[1,56]}$ = 5.4, P = 0.023). For juvenile rainbow trout the proportion of shallow

water fish found in edge habitat decreased from 0.26 ± 0.17 (n = 8) to 0.21 ± 0.15 (n = 8) in the presence of squawfish in the single-prey (low density) trials but increased from 0.20 ± 0.22 (n = 8) to 0.42 ± 0.25 (n = 8) in the two-prey (high density) trials. The response of adult roach was less affected by the presence of rainbow trout, with the proportion of shallow water fish found in edge habitat increasing slightly from 0.70 ± 0.45 (n = 5) to 0.87 ± 0.19 (n = 8) in the single-prey (low density) trials and staying unchanged at 1.00 ± 0.00 (n = 7) and 1.00 ± 0.00 (n = 8) in the two-prey (high density) trials.

Discussion

Our results support those of Brown and Moyle (1991) who documented changes in habitat choice among the resident fishes of the Eel River, California, in the presence of squawfish. In our study, habitat choice varied among the different prey studied. This was not surprising because field studies of California stream fishes have documented that roach and rainbow trout respond differently to a wide variety of environmental variables (Smith 1982; Taylor et al. 1982; Moyle and Daniels 1982; Brown and Moyle 1994). Other studies have documented differences in microhabitat use among species and size-classes (Moyle and Baltz 1985; Brown and Moyle 1991). Both this study and field data (Brown and Moyle 1991) indicate that roach and rainbow trout avoid deep water when squawfish are present but they tend to do so by moving to different shallow water habitats.

In our experiments each prey showed a very consistent response to the presence of squawfish (Fig. 2), with the response of each prey differing from that of the others. This consistency applies to both the proportion of total fish found in pool habitat and the distribution of the shallow water fish between edge and riffle habitat. These results conflict somewhat with the field results of Brown and Moyle (1991). Brown and Moyle (1991) found that the response of some species or size-classes varied between the South Fork Eel River and Van Duzen River and involved varying combinations of habitat and microhabitat shifts. They suggested that the sites differed in the types and amounts of refugia present, altering the level of predation risk. Work with other species has shown that individuals are able to assess risk of predation and adjust their behavior appropriately (Dill and Fraser 1984; Giles and Huntingford 1984; Fraser and Huntingford 1986; Fraser and Gilliam 1987; Harvey 1991; Dugatkin and Godin 1992). Also, there is variation among species in the level of risk that appears to be acceptable under identical experimental conditions (McLean and Godin 1989; Abrahams and Healey 1993). We purposely exposed the prey to a high level of risk, a high density of predatory squawfish, as the first step in assessing the importance of squawfish to determining the spatial structure of California stream fish assemblages. Our results show that with a consistent level of predation risk, a consistent level of response can be expected from the prey population. This lends credence to Brown and Moyle's (1991) suggestion that variation in habitat conditions between their two sites resulted in the variability in prey response they observed rather than random behavioral variability independent of habitat. In the stream, a wider range of prey responses should be expected because habitat heterogeneity is high, the density of predators is variable over time and space, and conditions affecting the success of a predator also vary (e.g., time of day, turbidity).

The results of the single-species trials suggest that the prey were balancing the perceived threat of predation from squawfish with the potential threat from avian and terrestrial predators. Juvenile roach completely avoided pool habitat when squawfish were present but adult roach and juvenile rainbow trout continued to use pool habitat to some extent. The tendency of larger fish to be found in deeper water than smaller fish has been attributed to the risk of predation from wading birds and mammals that prey on fish in shallow waters (Power 1987). Harvey and Stewart (1991) have documented this pattern in small eastern streams. Experiments conducted in the South Fork Eel River showed that adult roach and similar-sized fishes are highly vulnerable to predation by great blue herons (*Ardea herodias*) in shallow edge habitat (A.M. Brasher and L.R. Brown, unpublished data).

The two-prey experiments showed that adult roach and juvenile rainbow trout could each affect the habitat choice of the other. The presence of the other prey resulted in greater use of pool habitat where risk of predation was highest for both. The presence of the other prey also resulted in greater proportional use of edge habitat by shallow water fish of both prey. The effect was particularly interesting for rainbow trout because proportional use of edge habitat declined slightly (from 0.26 to 0.21) in the single-prey trials owing to the presence of squawfish but the proportion doubled (from 0.20 to 0.42) in the two-prey trials owing to the presence of squawfish. The mechanism causing these effects is unknown but possibilities include a simple density effect where the preferred refuge areas were filled and "excess" fish remained in the pool or shifted to the less preferred shallow water habitat. Alternatively, juvenile rainbow trout in the riffle area may have become more aggressive under the crowded conditions, excluding other fish to the other habitats. Our experiments were not adequate to distinguish between these possibilities; however, the latter mechanism seems more likely. California roach are not known to be territorial and often reach high densities in isolated pools (Moyle 1976); therefore, it seems unlikely that any intraspecies interaction would result in complete avoidance of riffle habitat in the two-prey trials. Aggressive behavior under crowded conditions by juvenile rainbow trout in their preferred riffle habitat could explain this result for adult roach and would also be consistent with greater use of edge and pool habitat by the remaining juvenile rainbow trout.

Whatever the mechanism, these results may be applicable to several field situations. The experimental design was meant to simulate a situation where a predator moves into a predator-free area and prey fish are forced to concentrate in a restricted refuge area. The results may also reflect the situation when abundant larval fish reach sufficient size to become potential prey for large squawfish, where occurrence of squawfish is seasonal, or when overall squawfish densities are low and occurrence at a specific location may be sporadic. For example, our results

suggest that risk of predation for juvenile rainbow trout in the Eel River, which is presently being invaded by squawfish (Brown and Moyle 1991), may be high because spatial overlap with other species including adult roach has increased as resident fish crowd into limited refuge habitat. Such interactions may not be important where refuge habitat is abundant or prey densities are maintained at low levels by other factors such as fluctuations in abiotic variables.

Baltz and Moyle (1993) used null models to assess the importance of biotic interactions to the ability of assemblages of native California stream fishes to resist invasion by introduced fishes. Even though there was noticeable interspecific microhabitat segregation among species in the native fish assemblage, the null model results indicated more spatial overlap than would be expected at random. They suggested that the observed spatial structure of the assemblage was due to a combination of evolutionary events, predation by Sacramento squawfish, and competition, probably in decreasing order of importance. They also suggested that predator-driven species interactions, such as those we observed during the two-prey experiments, may be an important effect of the presence of squawfish. As interest in preserving aquatic biodiversity increases, the need to understand the interplay among the many interacting processes determining the characteristics of native stream fish assemblages will also increase. Further studies of native and introduced predators, including all species of squawfish, will be vital to developing the knowledge necessary to preserve native stream fish assemblages.

Acknowledgements

We thank John Church, William Coles, Tom Kennedy, and Derek Kuda for help running the experiments. Bill Bennett, Laura Rogers-Bennett, John McGrath, and Rollie White helped construct and install the streams. John McGrath and the Garberville Rotary Club provided access to the site. Peter B. Moyle, Terry M. Short, Bret C. Harvey, and the U.C. Davis Fish Ecology Research Collective reviewed and improved several early versions of the manuscript. Comments by two reviewers significantly improved the final version. This work was supported, in part, by Dingell-Johnson/Wallop-Breaux Federal Aid in Sport Fish Restoration Act funds (California Project F-51-R, Subproject IX, Study 8, Jobs 2–6), administered through the California Department of Fish and Game.

References

Abrahams, M.V., and M.C. Healey. 1993. A comparison of the willingness of four species of Pacific salmon to risk exposure to a predator. Oikos, 66: 439–446.

Angermeier, P.L. 1992. Predation by rock bass on other stream fishes: experimental effects of depth and cover. Environ. Biol. Fishes, 34: 171–180.

Baltz, D.M., and P.B. Moyle. 1993. Invasion resistance to introduced species by a native assemblage of California stream fishes. Ecol. Appl. 3: 246–255.

Brown, L.R. 1991. Age, growth, feeding, and behavior of Sacramento squawfish (*Ptychocheilus grandis*) in Bear Creek, Colusa County, California. Southwest. Nat. 35: 249–260.

Brown, L.R., and P.B. Moyle. 1981. The impact of squawfish on salmonid populations: a review. N. Am. J. Fish. Manage. 1: 104–111.

Brown, L.R., and P.B. Moyle. 1991. Changes in habitat and microhabitat partitioning within an assemblage of stream fishes in response to predation by Sacramento squawfish (*Ptychocheilus grandis*). Can. J. Fish. Aquat. Sci. 48: 849–856.

Brown, L.R., and P.B. Moyle. 1994. Distribution, ecology, and status of the fishes of the San Joaquin River drainage, California. Calif. Fish Game, 79: 96–114.

Cerri, R.D., and D.F. Fraser. 1983. Predation and risk in foraging minnows: balancing conflicting demands. Am. Nat. 121: 552–561.

Dill, L.M., and A.H.G. Fraser. 1984. Risk of predation and the feeding behavior of juvenile coho salmon (*Oncorhynchus kisutch*). Behav. Ecol. Sociobiol. 16: 65–71.

Dugatkin, L.A., and J.-G.J. Godin. 1992. Predator inspection, shoaling and foraging under predation hazard in the Trinidadian guppy, *Poecilia reticulata*. Environ. Biol. Fishes, 34: 265–276.

Fraser, D.F., and E.E. Emmons. 1984. Behavioral response of blacknose dace (*Rhinichthys atratulus*) to varying densities of predatory creek chub (*Semotilus atromaculatus*). Can. J. Fish. Aquat. Sci. 41: 364–370.

Fraser, D.F., and J.F. Gilliam. 1987. Feeding under predation hazard: response of the guppy and Hart's rivulus from sites with contrasting predation hazard. Behav. Ecol. Sociobiol. 21: 203–209.

Fraser, D.F., and F.A. Huntingford. 1986. Feeding and avoiding predation hazard: the behavioral response of the prey. Ethology, 73: 56–68.

Giles, N., and F.A. Huntingford. 1984. Predation risk and interpopulation variation in anti-predator behaviour in the three-spined stickleback, *Gasterosteus aculeatus* L. Anim. Behav. 32: 264–275.

Harvey, B.C. 1991. Interactions among stream fishes: predator-induced habitat shifts and larval survival. Oecologia, 87: 29–36.

Harvey, B.C., and A.J. Stewart. 1991. Fish size and habitat depth relationships in headwater streams. Oecologia, 87: 336–342.

McLean, E.B., and J.-G.J. Godin. 1989. Distance to cover and fleeing from predators in fish with different amounts of defensive armour. Oikos, 55: 281–290.

Moyle, P.B. 1976. Inland fishes of California. University of California Press, Berkeley, Calif.

Moyle, P.B. 1986. Fish introductions into North America: patterns and ecological impact. *In* Ecology of biological invasions of North America and Hawaii. *Edited by* H.A. Mooney and J.A. Drakes. Springer Verlag, New York. pp. 27–43.

Moyle, P.B., and D.M. Baltz. 1985. Microhabitat use by an assemblage of California stream fishes: developing criteria for instream flow determinations. Trans. Am. Fish. Soc. 114: 695–704.

Moyle, P.B., and R.A. Daniels. 1982. Fishes of the Pit River system, McCloud River system, and Surprise Valley region. Univ. Calif. Publ. Zool. 115: 1–82

Moyle, P.B., H.W. Li, and B. Barton. 1986. The Frankenstein effect: impact of introduced fishes on native fishes in North America. *In* Fish culture in fisheries management. *Edited by* R.H. Stroud. American Fisheries Society, Bethesda, Md. pp. 415–426.

Power, M.E. 1987. Predator avoidance by grazing fishes in temperate and tropical streams: importance of stream depth and prey size. *In* Predation: direct and indirect impacts on aquatic communities. *Edited by* W.C. Kerfoot and A. Sih. University Press of New England, Hanover, N.H. pp. 333–351.

Power, M.E., W.J. Matthews, and A.J. Stewart. 1985. Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. Ecology, 66: 1448–1456.

Rieman, B.E., R.C. Beamsderfer, S. Vigg, and T.P. Poe. 1991. Estimated loss of juvenile salmonids to predation by northern squawfish, walleyes, and smallmouth bass in John Day Reservoir, Columbia River. Trans. Am. Fish. Soc. 120: 448–458.

Schlosser, I.J. 1987. The role of predation in age- and size related habitat use by stream fishes. Ecology, 68: 651–659.

Schlosser, I.J. 1988. Predation rates and behavioural response of adult brassy minnows (*Hybognathus hankinsoni*) to creek chub and smallmouth bass predators. Copeia, 1988: 691–697.

Schlosser, I.J., and K.K. Ebel. 1989. Effects of flow regime and cyprinid predation on a headwater stream. Ecol. Monogr. 59: 41–57.

Smith, J.J. 1982. Fishes of the Pajaro River system. Univ. Calif. Publ. Zool. 115: 85–169.

Taylor, T.L., P.B. Moyle, and D.G. Price. 1982. Fishes of the Clear Lake basin. Univ. Calif. Publ. Zool. 115: 171–224.

Vigg, S., T.P. Poe, L.A. Prendergast, and H.C. Hansel. 1991. Rates of consumption of juvenile salmonids and alternative prey fish by northern squawfish, walleyes, smallmouth bass, and channel catfish in John Day Reservoir, Columbia River. Trans. Am. Fish. Soc. 120: 421–438.

Comparative energetics and the development of bioenergetics models for sympatric estuarine piscivores

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Abstract: Bioenergetics models for striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*), and weakfish (*Cynoscion regalis*) were developed from laboratory experiments on metabolism and consumption. Size-specific rates of consumption and metabolism were similar for bluefish and weakfish and higher than those for striped bass. Temperature effects on maximum consumption rate (C_{max}) differed with fish size. C_{max} of young fish (30 g) increased with temperature, then declined rapidly at higher temperatures; C_{max} for larger fish of all three species (100–3000 g) increased rapidly to the maximum rate, but leveled off at higher (25–30°C) temperatures. Results of C_{max} experiments suggest that extrapolation of the temperature dependency of small fish to larger fish, as is commonly done, may misrepresent potential growth at higher temperatures. Independent model validation using laboratory experiments found consumption estimates (from growth) to be within −1.4 to +4.5% of known values for all species at temperatures above 19°C; however, at 6.9°C consumption by striped bass was overestimated by 20–46%. Model estimates of growth (from consumption) were within −7.1 to +30.1% of known values in all validations. Overall, the growth physiology of the three species appeared to be related to the water temperatures encountered during estuarine residency and production.

Résumé : Des modèles décrivant la bioénergétique du bar d'Amérique (*Morone saxatilis*), du tassergal (*Pomatomus saltatrix*) et de l'acoupa royal (*Cynoscion regalis*) ont été construits à partir de travaux en laboratoire sur leur métabolisme et sur leur consommation alimentaire. On a observé que chez le tassergal et l'acoupa royal, le métabolisme et la consommation atteignent des taux en fonction de la taille semblables entre eux et supérieurs à ceux mesurés chez le bar d'Amérique. Les effets de la température sur la consommation maximale diffèrent selon la taille des poissons. Chez les jeunes (30 g), la consommation maximale s'accroît avec la température pour s'abaisser rapidement passé un certain plafond. Peu importe les espèces, elle s'accroît rapidement pour atteindre son maximum chez les sujets les plus gros (100 à 300 g), mais se stabilise entre 25 et 30°C. Nos expériences paraissent indiquer que l'extrapolation aux poissons de grande taille de la relation observée entre la consommation maximale et la température chez les poissons de petite taille, comme cela se fait couramment, peut constituer une représentation faussée de la croissance potentielle en eau chaude. La validation indépendante par des modèles à des expériences en laboratoire a montré que les estimations de la consommation alimentaire (à partir de la croissance) se situent entre −1,4 et +4,5% des valeurs connues pour toutes les espèces à des températures dépassant 19°C; toutefois, à 6,9°C, la consommation du bar d'Amérique est surestimée de 20 à 46%. Les estimations de la croissance (mesurée à partir de la consommation) obtenues par modélisation se situent entre −7,1 et +30,1% des valeurs déterminées par l'ensemble des tests de modélisation. Dans l'ensemble, la physiologie de la croissance chez les trois espèces paraît être en rapport avec la température de l'eau pendant la période de résidence et de production en estuaire.

[Traduit par la Rédaction]

Received November 17, 1994. Accepted March 3, 1995.
J12164

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Introduction

Striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*), and weakfish (*Cynoscion regalis*) support valuable recreational and commercial fisheries throughout their range along much of the east coast of the United States (Wilk 1979; Mercer 1983; Mid-Atlantic Fishery Management Council et al. 1989). These species also represent the top piscivores in many estuarine systems (Manooch 1973; Merriner 1975; Chao and Musick 1977; Gardinier and Hoff 1982; Friedland et al. 1988) and should play a key ecological role either by influencing lower trophic levels (top-down control) or by occupying the top end of ecosystem production (bottom-up control, Carpenter et al. 1985, 1987).

Striped bass, bluefish, and weakfish differ taxonomically, spanning three families of fishes (Percichthyidae, Pomatomidae, and Scianidae, respectively), and thus provide an interesting ecological contrast. Bluefish are highly mobile pelagic predators (Olla and Studholme 1971, 1972), weakfish are considered to be more demersal predators, often associated with deep channel habitats (Chao and Musick 1977; Wilk 1979; Mercer 1983), and striped bass are reported to use both strategies (Setzler et al. 1980). The three species also differ in their estuarine residency patterns. Striped bass, bluefish, and weakfish each use the Chesapeake Bay estuary as a nursery area. However, striped bass are annual residents of the Chesapeake Bay until age 5 or 6 when they become coastal migrants (Kohlenstein 1981). In contrast, bluefish and weakfish migrate into the estuary in spring and emigrate in fall (Norcross et al. 1974; Chao and Musick 1977; Kendall and Walford 1979). The migratory patterns of bluefish and weakfish may have important ecological significance. Immigration of these species and their potential predatory impact is difficult to predict or manage because much of predator growth and mortality is decoupled from the prey supply within the Chesapeake Bay as rates of immigration are also determined by physical factors. This decoupling of piscivore growth and mortality from prey supply may prevent equilibration between predator demand and prey production or supply.

Because fish are cold blooded their predatory capacity is regulated largely by environmental temperature (Brett and Groves 1979). Temperature constrains predation rates by regulating the rate at which food is digested (Swenson and Smith 1973; Elliott and Persson 1978), the rate of metabolism (Beamish 1970), the swimming speed (Olla and Studholme 1971; Stewart et al. 1983), and rates of egestion and excretion (Elliott 1976; Kitchell et al. 1977). Because of the ecological and taxonomic differences among striped bass, bluefish, and weakfish, physiological differences in digestion and metabolic responses to temperature also are probable.

Recently, bioenergetics models have emerged as a useful tool in predicting the predation rates of fish (Kitchell et al. 1977; Kitchell and Breck 1980; Stewart et al. 1981, 1983; Stewart and Ibarra 1991; Hartman and Margraf 1992) and in comparing predation and energetics across species (Moore 1988; Stewart and Ibarra 1991). Bioenergetics models rely on the balanced energy equation of Winberg (1956):

$$[1] \quad G = C - (R + \mathrm{SDA}) - F - U$$

where all units are in energy and G is growth, C is consumption, R is metabolism, SDA is specific dynamic action or the heat increment (associated with biochemical transformation of ingested food into metabolizable form), F is egestion, and U is excretion. In the balanced energy equation, C and R are modeled as functions of both temperature and body size. Metabolism is often further divided into resting or standard metabolism (R_s) and active metabolism (R_{act}).

In this paper, we assemble a complete bioenergetics model based on experiments and the literature for striped bass, bluefish, and weakfish. We conducted laboratory experiments on the effects of temperature and fish size on maximum consumption and routine metabolism. From these data, we developed bioenergetics models for each species, validated the models in the laboratory using independent experiments, and finally compared the bioenergetics and scope for growth, among striped bass, bluefish, and weakfish.

Material and methods

General procedures

Experimental fish were collected from a variety of sources in the mid-Chesapeake Bay and the lower Delaware Bay. Age-0 striped bass (Patuxent River, Maryland, stock) were obtained from the Maryland Department of Natural Resource's Manning Fish Hatchery during July 1990, and from the Potomac Electric Power Company's Chalk Point aquaculture facility during October 1991. Age-1 and older striped bass were collected as needed by angling at the mouth of the Patuxent River, Maryland, during 1990–1992 and at Calvert Cliffs, Maryland, during spring 1992. Bluefish were collected by angling in the mid-Chesapeake Bay during summer 1991 and 1992. Age-0 weakfish were obtained from the lower Delaware River during summer 1992 by using a beach seine. Age-1 and older weakfish were obtained by angling during late summer 1992 at the mouth of the Patuxent River. All experiments were conducted at Chesapeake Biological Laboratory's Controlled Environmental Laboratory between October 1990 and March 1993. Experiments involving striped bass were conducted throughout the year to include all possible temperatures encountered during estuarine residency. Experiments with bluefish and weakfish were restricted to the May through December periods of each year. We used filtered (5 μm) Patuxent River water with a mean salinity of 15‰ (range, 11–17‰) for all experiments.

Care was taken to ensure that fish were properly acclimated prior to experimentation. Only fish that had been held in captivity for a minimum of 4 weeks and judged to be behaving normally (i.e., were feeding and orienting themselves like other fishes under the same conditions) were used in experiments. For all experiments except those at 29°C, fish were held in flow-through tanks to which ambient Patuxent River water was provided. Photoperiod matched natural hours of daylight:darkness (e.g., 15 h light : 9 h dark during July) and photoperiods were adjusted monthly to new ambient daylight:darkness periods. When ambient Patuxent River water temperatures reached those

chosen for experiments, fish were acclimated to test tanks for 2–4 d prior to beginning experiments. We were forced to heat ambient water in the 29°C experiments because ambient temperatures did not exceed 27°C during either 1991 or 1992. For the 29°C experiments, fish were acclimated from 25 to 29°C for 3 weeks prior to experimentation. Photoperiod (light:dark) ranged from 9:15 at 5°C, to 15:9 at 25°C, to 14:10 at 29°C.

Before experiments, striped bass were fed either commercial trout chow (Ziegler Brothers, Inc., 5/32-inch Trout Grower), frozen bay anchovy (*Anchoa mitchilli*), frozen Atlantic menhaden (*Brevoortia tyrannus*), or live *Fundulus* spp. once per day. Bluefish and weakfish would not accept commercial trout chow and were fed only frozen bay anchovy or Atlantic menhaden, or live *Fundulus* spp. once per day.

At the beginning of each experiment, fish were fasted for 24 h to standardize time for gastric evacuation. Fish were anesthetized with MS-222, weighed (to ±0.1 g wet weight), and allowed to acclimate in experimental tanks an additional 24 h for metabolism experiments and 8–16 h for consumption experiments. Fish were also anesthetized and weighed (±0.1 g wet weight) at the conclusion of all experiments to estimate net growth during the experiment. The conclusion of respirometry experiments was considered to be immediately following the last observation. Maximum consumption experiments were ended with a final weight determination of fish 24 h after the last feeding to standardize gastric fullness between initial and final weights.

Maximum consumption methods

The effect of temperature and fish weight upon the maximum consumption (C_{max}) was evaluated using a range of experimental temperatures (2–30°C) and fish sizes (5–2700 g). The experimental set-up for maximum consumption experiments varied depending upon the size of the fish. Small fish (5–60 g) were placed individually into 38-L aquaria, whereas larger fish (>61 g) were placed in groups of two or three in 500-L circular tanks for experimentation. Groups of two to three fish were used in C_{max} experiments with larger fish because individuals failed to feed in isolation. In experiments with small fish we used individuals, which provided a measure of the variability in C_{max} among and within individuals. Use of individual small fish in C_{max} experiments was justified because preliminary experiments showed no significant differences in maximum consumption for individuals versus two or three fish for small striped bass, bluefish, or weakfish at 24°C (K. Hartman, unpublished data).

To evaluate temperature effects on C_{max} of age-0 striped bass (10–59 g wet weight, mean 39.3 g), individual fish were placed into 38-L aquaria and fed thawed bay anchovy ad libitum at temperatures of 3, 7, 13, 18, 23, 27, and 30°C with 12–24 replicates/temperature. We examined age-0 weakfish (5–61 g wet weight, mean 26.2 g) at 15, 20, 24, and 28°C with 5–11 replicates/temperature.

Experiments to determine the effects of temperature on larger fish (>61 g) were conducted in 500-L circular tanks. C_{max} experiments were replicated 4–12 times/temperature for bluefish (100–965 g) at 10, 15, 20, 24, and 29°C. For striped bass (61–2700 g) C_{max} experiments were run at 6, 13, 15, 20, 23, 25, 27, and 29°C with 4–8 replicates/temperature. Estimates of C_{max} for larger weakfish were replicated 3–10 times each at 15, 20, 25, and 29°C.

In all C_{max} experiments, each replicate was duplicated (same individual fish) for 3–7 consecutive d to arrive at mean maximum consumption levels. Temperatures were recorded and fish were fed thawed bay anchovy or Atlantic menhaden ad libitum twice each d at 08:00 and 18:00 h for 3–7 days. Bay anchovy and Atlantic menhaden were chosen as prey because they represent common natural foods of these predators in the Chesapeake Bay (Hartman 1993; Hartman and Brandt 1995*a*). The twice-daily feeding regime was selected because striped bass have been reported to be crepuscular feeders (Raney 1952), and because preexperiment trials with striped bass, bluefish, and weakfish showed no significant differences in maximum consumption when fish were fed 2 or 4 times during the 24-h cycle. Consumption was estimated as the difference between the wet weight (after blotting) of food offered and the wet weight recovered after 1 h. Consumption during both feedings was summed to determine daily specific consumption, C_{max} ($g \cdot g^{-1} \cdot d^{-1}$).

The relationship between fish size and C_{max} is needed to compare the effects of temperature on C_{max} across species (used to standardize experimental data to a particular sized fish) and to define the dependency of C_{max} on fish size. The effect of fish size on consumption can be described by the allometric equation $Y = CA \cdot W^{CB}$, where Y is the specific rate of consumption ($g \cdot g^{-1} \cdot d^{-1}$), W is body weight, and CA and CB are species-specific constants (Brett 1971; Kitchell et al. 1977; Brett and Groves 1979; Stewart et al. 1981, 1983; Stewart and Binkowski 1986). We used the allometric equation to describe size-effects on C_{max}. The allometric equation (power function) explained more of the variance (r^2) in simple regression models than those incorporating linear, exponential, or $\log_{10}$ functions. Further, the allometric equation is also the form commonly used in a common bioenergetics software package and therefore this form may be most useful to other investigators. In estimating the allometric variables for each species we used only data from experiments done at 20–25°C because these temperatures encompassed maximum consumption rates for these fishes. We used linear least-squares regressions to generate best fits of the allometric variables to the species-specific C_{max} data. The allometric exponent (CB) for each species was then used to standardize the experimental C_{max} data to specific sizes for comparison of C_{max} across temperature and species. The complete allometric equation ($CA \cdot W^{CB}$) was later used to describe the size dependency of C_{max} in bioenergetics models for each species (Tables 1–3).

Maximum consumption data were standardized for 30-, 100-, 350-, and 1000-g fish for comparisons across species. Because the temperature dependency of C_{max} may vary with ontogeny, we used only portions of the C_{max} data to model each size grouping. We used field-observed size-at-age ranges as the basis for partitioning of the C_{max} data. For example, in field situations a 30-g striped bass would be of age-0. Therefore, only C_{max} data from fish of sizes commonly attained by age-0 striped bass in the field (i.e., 6–59 g, Hartman 1993) were included in the 30-g striped

Table 1. Symbols and estimated parameter values used in a bioenergetics model for striped bass.

	Parameter description	Parameter values
	Consumption, C_{max}	
CA	Intercept: C_{max} at $(\Theta_2 + \Theta_3)/2$	0.3021 ± 0.1081
CB	Coefficient: C_{max} versus weight	−0.2523 ± 0.082
Θ_1	Temperature for K_1 (Thornton and Lessem 1978) (age 0, age 1, age 2, age ≥ 3)	2.6, 6.6, 6.6, 7.4
Θ_2	Temperature for K_2 (Thornton and Lessem 1978) (age 0, age 1, age 2, age ≥ 3)	21.6, 19.0, 18.0, 15.0
Θ_3	Temperature for K_3 (Thornton and Lessem 1978) (age 0, age 1, age 2, age ≥ 3)	22.7, 28.0, 29.0, 28.0
Θ_4	Temperature for K_4 (Thornton and Lessem 1978) (age 0, age 1, age 2, age ≥ 3)	28.3, 30.0, 32.0, 30.0
K_1	Proportion of C_{max} at Θ_1 (age 0, age 1, age 2, age ≥ 3)	0.047, 0.262, 0.255, 0.323
K_2, K_3	Proportion of C_{max} at Θ_2, Θ_3 (all ages)	0.98
K_4	Proportion of C_{max} at Θ_4 (age 0, age 1, age 2, age ≥ 3)	0.713, 0.850, 0.900, 0.850
	Metabolism, *R*	
RA	Intercept: R (g $O_2 \cdot d^{-1}$) (age 0, age ≥ 1)	0.001 456 ± 0.003, 0.002 80 ± 0.0005
RB	Coefficient: R versus weight (age 0, age ≥ 1)	−0.2702 ± 0.1137, −0.218 ± 0.0310
RQ	Coefficient: R versus temperature (age 0, age ≥ 1)	0.083 39 ± 0.0056, 0.0760 ± 0.0054
SDA	Coefficient: specific dynamic action (proportion of assimilated food)	0.172[a]
ACT	Coefficient: multiplier of metabolism (age-0, age ≥ 1)	2.463 ± 0.429, 1.649 ± 0.206
	Egestion, *F*	
F	Proportion of consumed food egested	0.104[b]
	Excretion, *E*	
E	Proportion of assimilated food excreted	0.068[b]

Note: Parameters are for equations in Thornton and Lessem (1978), Stewart and Binkowski (1986), and Hewett and Johnson (1992), but see Appendix. Parameter values were determined in the present study unless otherwise noted and are given ± 95% confidence intervals, where appropriate. Multiple values for a variable represent values for age-0 followed by age-1, and by age-2 and (or) older fish.

[a]Hewett and Johnson (1992).

[b]Rice et al. (1983).

bass C_{max} standardization. The same field size-at-age criteria were used for other sizes and species standardizations (Table 4).

Maximum consumption results

The maximum weight-specific consumption rate declined with increasing fish size for all three species (Fig. 1). Bluefish had the highest allometric intercept (0.520) and the largest exponent (−0.288) of the three species. Striped bass allometry had the lowest intercept (0.302) and the smallest exponent (−0.252). Weakfish allometric variables were intermediate to those of the other species (Fig. 1). Coefficients of determination (r^2) for regression equations describing C_{max} as a function of fish size were 0.60 for bluefish, 0.77 for striped bass, and 0.87 for weakfish. Slopes for size-dependent consumption functions were not significantly different among the three species (ANCOVA, $P > 0.01$). However, the intercept for size dependence was significantly different between striped bass and the other two species. No significant differences in the size dependence of C_{max} were found between bluefish and weakfish.

Temperature had a positive effect on the C_{max} of all species (Fig. 2). C_{max} of small (30 g) striped bass increased with temperature up to 23°C, but consumption rates declined above 23°C (Fig. 2). C_{max} values for 30-g striped bass varied greatly (0.07–0.18 $g \cdot g^{-1} \cdot d^{-1}$) among individuals at 23°C. C_{max} of small weakfish also increased with temperature from 15 through 24°C, but declined between 24 and 29°C (Fig. 2). C_{max} of small bluefish appeared to increase with temperature from 10 through 26°C, the highest temperature for which data were available (Fig. 2).

Maximum consumption increased with temperature for 100-, 350-, and 1000-g fish, but rates of increase with temperature appeared to be more rapid for smaller fish than for larger ones. C_{max} generally increased with increasing temperature; however, with increasing fish size C_{max} did not rapidly decline with increasing temperature beyond 20°C as was observed for 30-g fish (Fig. 2). In general, the degree of temperature dependency appeared to decline with increasing fish size. The C_{max} for a 1000-g striped bass was nearly constant from 20–30°C (Fig. 2), whereas the range of temperatures of constant, high C_{max} declined to 22–28°C for 350-g striped bass (Fig. 2). For all sizes of bluefish, C_{max} generally increased with temperature through 26 or 30°C. In contrast, the temperature dependency of C_{max} for 30-g and 100-g striped bass and weakfish was sharper, with a narrower range of temperatures at which peak C_{max} occurred than for fish larger than 100 g (Fig. 2).

Species-specific differences in C_{max} were apparent across fish sizes and temperatures. In comparisons across species for 30-, 100-, 350-, and 1000-g predators (ANCOVA, $P >$

Table 2. Symbols and estimated parameter values used in a bioenergetics model for age-0 through age-2 bluefish.

	Parameter description	Parameter values
	Consumption, C_{max}	
CA	Intercept: C_{max} at $(\Theta_2 + \Theta_3)/2$	0.5197 ± 0.2907
CB	Coefficient: C_{max} versus weight	−0.288 ± 0.142
Θ_1	Temperature for K_1 (Thornton and Lessem 1978)	10.2
Θ_2	Temperature for K_2 (Thornton and Lessem 1978)	23.0
Θ_3	Temperature for K_3 (Thornton and Lessem 1978)	28.0
Θ_4	Temperature for K_4 (Thornton and Lessem 1978)	32.0
K_1	Proportion of C_{max} at Θ_1	0.156
K_2, K_3	Proportion of C_{max} at Θ_2, Θ_3	0.980
K_4	Proportion of C_{max} at Θ_4	0.850
	Metabolism, *R*	
RA	Intercept: R (g $O_2 \cdot d^{-1}$)	0.005 58 ± 0.000 68
RB	Coefficient: R versus weight	−0.264 ± 0.126
RQ	Coefficient: R versus temperature	0.069 25 ± 0.0160
SDA	Coefficient: specific dynamic action (proportion of assimilated food)	0.172[a]
ACT	Coefficient: multiplier of metabolism	1.8805 ± 0.328
	Egestion, *F*	
F	Proportion of consumed food egested	0.104[b]
	Excretion, E	
E	Proportion of assimilated food excreted	0.068[b]

Note: Parameters are for equations in Thornton and Lessem (1978), Stewart and Binkowski (1986), and Hewett and Johnson (1992), but see Appendix. Parameter values were determined in the present study unless otherwise noted and are given ± 95% confidence invervals, where appropriate.

[a]Hewett and Johnson (1992).

[b]Rice et al. (1983).

0.01), striped bass C_{max} was always significantly different from that of bluefish or weakfish. For small (30 and 100 g) bluefish and weakfish, C_{max} was significantly different, but for larger fish (350 and 1000 g) only the intercepts differed significantly. Across species, bluefish generally had higher C_{max} values than weakfish or striped bass at a given temperature for each predator size (Fig. 2). For example, bluefish C_{max} for 30-g fish at 20°C averaged 0.16 $g \cdot g^{-1} \cdot d^{-1}$, while striped bass and weakfish C_{max} averaged 0.09 and 0.04 $g \cdot g^{-1} \cdot d^{-1}$, respectively. Similarly, C_{max} of 1000-g bluefish averaged 0.07 $g \cdot g^{-1}\ d^{-1}$ from 20-25°C, while the values for striped bass (0.04 $g \cdot g^{-1} \cdot d^{-1}$) and weakfish (0.06 $g \cdot g^{-1} \cdot d^{-1}$) were considerably lower. Striped bass usually had lower C_{max} at a given temperature than did similar-sized bluefish or weakfish. Weakfish C_{max} was lower than that of striped bass or bluefish at lower temperatures (around 15°C), but was within 14% of that of bluefish for temperatures between 20 and 25°C (Fig. 2).

Routine metabolism methods

Routine metabolism of striped bass, bluefish, and weakfish was measured on the same fishes used in the consumption experiments. Acclimation periods for each temperature were 8–21 d. Metabolism experiments on small fish (<60 g) were done in 3-L Fernbach flasks, whereas experiments on medium fish (60–600 g) and large fish (>600 g) were done in 135- and 310-L tanks, respectively. For the small fish, a 129-L tank served as a bath for the six 3-L flasks to maintain a constant temperature. A pump returned water from the bath tank to a 70-L headbox (under aeration), which then provided water to each flask. Flow rates to each flask were regulated to achieve a 1.0–2.0 $mg \cdot L^{-1}$ differential between inflow and outflow oxygen levels. Individual fish were placed into the Fernbach flasks, which had previously been masked with black plastic to minimize disturbances to the fish during measurements. Dissolved oxygen (DO) concentrations were measured (±0.01 $mg \cdot L^{-1}$) at the inflow and outflow with a YSI model 58 DO meter. The DO meter was air calibrated before each measurement to ensure accurate measurements of oxygen differential. The rate of oxygen consumption ($mg \cdot g^{-1} \cdot h^{-1}$) was calculated as the product of oxygen differential and flow rate and standardized per unit fish weight (Zastrow and Houde 1990).

Metabolism experiments for medium and large fish were conducted differently. Metabolism for the medium and large fish was measured in sealed tanks that allowed the fish to deplete oxygen levels over time. Fish were placed individually

Table 3. Symbols and estimated parameter values, used in bioenergetics models for age-0 and age-1 and older weakfish.

	Parameter description	Parameter values
	Consumption, C_{max}	
CA	Intercept: C_{max} at $(\Theta_2 + \Theta_3)/2$	0.492 ± 0.030
CB	Coefficient: C_{max} versus weight	−0.2680 ± 0.014
Θ_1	Temperature for K_1 (Thornton and Lessem 1978)	14.87, 14.80
Θ_2	Temperature for K_2 (Thornton and Lessem 1978)	24.3, 25.0
Θ_3	Temperature for K_3 (Thornton and Lessem 1978)	24.3, 25.0
Θ_4	Temperature for K_4 (Thornton and Lessem 1978)	27.7, 29.0
K_1	Proportion of C_{max} at Θ_1	0.0334, 0.195
K_2, K_3	Proportion of C_{max} at Θ_2, Θ_3	0.98
K_4	Proportion of C_{max} at Θ_4	0.561, 0.970
	Metabolism, *R*	
RA	Intercept: R (g $O_2 \cdot d^{-1}$)	0.0009 ± 0.00001, 0.0030 ± 0.0005
RB	Coefficient: R versus weight	−0.1254 ± 0.0108, −0.155 ± 0.0240
RQ	Coefficient: R versus temperature	0.0912 ± 0.0014, 0.0508 ± 0.0025
SDA	Coefficient: specific dynamic action (proportion of assimilated food)	0.172[a]
ACT	Coefficient: multiplier of metabolism	3.430 ± 0.530, 2.465 ± 0.721
	Egestion, *F*	
F	Proportion of consumed food egested	0.104[b]
	Excretion, *E*	
E	Proportion of assimilated food excreted	0.068[b]

Note: Parameters are for equations in Thornton and Lessem (1978), Stewart and Binkowski (1986), and Hewett and Johnson (1992), but see Appendix. Parameter values were determined in the present study unless otherwise noted and are given ± 95% confidence invervals, where appropriate. Multiple values for a variable represent values for age-0 followed by age-1 and older models.

[a]Hewett and Johnson (1992).

[b]Rice et al. (1983).

into either one of three 135- or 310-L circular tanks. Each tank was fitted with a fiberglass cover and rubber gasket that was bolted onto the tank to ensure that there was no exchange of oxygen with the air. Water was provided to each tank through a hose in the lids. A standpipe inside each tank and a valve on the outflow (outside each tank) allowed water flow to be regulated in metabolic chambers, changing from a flow-through system (used during acclimation and between metabolism measurements) to a closed system during metabolism measurements. DO and temperature were measured at the beginning of an observation and again after allowing sufficient time for the fish to deplete oxygen levels inside the tank by 1–2 mg·L^{-1} (0.8–4.5 h, depending upon temperature and fish size). All measurements of DO and temperature were made as for small fish.

Preliminary measurements (for each species and fish size) over 4 d showed that routine metabolism declined to a constant level 16–20 h after fish were placed in the metabolic chambers (flasks or tanks). On the basis of these observations, fish were allowed to acclimate to the metabolic chambers for 24 h prior to experimentation. All measurements were made between 24 and 48 h after fish were placed into the metabolic chamber. Control observations on empty metabolic chambers at each temperature showed no detectable change in DO levels within the chambers in the absence of fish.

Metabolic experiments were conducted over a wide range of fish sizes and temperatures for each species. Metabolism of small striped bass was replicated with 12–24 individual fish at each of 6, 10, 16, 23, 27, and 30°C. Metabolism of larger striped bass (>61 g) was replicated 6–19 times each at 7.5, 13, 15, 18.5, 20, 23.5, 26, and 29°C. Metabolic experiments for bluefish (all >60 g) were replicated 8–16 times at 15, 19.5, 25, and 29°C. Metabolism for small weakfish was replicated 9–16 times at 12, 17, 22, and 25°C. Metabolic experiments for larger weakfish (>60 g) were replicated 5–11 times at 15.5, 19, 24, and 29°C.

The routine metabolism data were used to develop least-squares models of metabolism as functions of temperature and fish size for incorporation into bioenergetics models. The allometric exponent (RB) from each of these equations was then used to standardize the laboratory metabolism data to 30, 100, 350, and 1000 g in a similar manner to that for C_{max}, so that comparisons of the temperature effects across sizes and species could be made.

Table 4. Size ranges of fish (g) and numbers of observations (N) for each standardized size used to compare C_{max} and routine metabolism of striped bass, bluefish, and weakfish.

	Striped bass		Bluefish		Weakfish	
Size[a]	Range	N	Range	N	Range	N
			C_{max}			
30	10–60	115	26–293	18	6–60	37
100	21–423	24	26–567	32	33–279	24
350	189–961	34	89–686	33	148–431	13
1000	547–2742	26	217–1013	31	148–1251	14
			Routine metabolism			
30	7–81	86	19–497	42	5–58	50
100	60–496	53	19–497	42	108–299	18
350	156–858	64	19–497	42	160–480	22
1000	405–2886	81	402–1013	40	210–1318	22

[a]Observations of C_{max} or routine metabolism that fell within the size range for each weight class (30, 100, 350, or 1000 g) were standardized to the weight class size by using the weight-dependent exponents for each species for comparisons across species and temperatures.

We observed that for striped bass and weakfish, there was a significant effect of metabolic chamber type on the measurements of routine metabolism. Analysis of covariance showed significant interaction ($P < 0.0001$) between metabolic chamber type (tank or flask) and the weight- and temperature-specific rate of metabolism. Least-squares regression solutions for routine metabolism of striped bass and weakfish that included both respirometer types, linearized by $\log_e$–$\log_e$ transformation, showed no significant size-dependence effect, although one was expected. Therefore, we separated the metabolic data that were obtained from flask experiments from that obtained from tanks and constructed least-squares regression solutions for each respirometer type and species independently. Data from the flask-type respirometers were used in constructing the age-0 striped bass or weakfish models, whereas data from the tank-type respirometers were used in constructing the age-1 and older models.

We modeled metabolism as a function of wet weight (allometric equation) and temperature (exponential) as is normally done (Rice et al. 1983; Stewart and Binkowski 1986; Rudstam 1988; Wahl and Stein 1991). We also modeled routine metabolism as a multiple regression model with temperature and wet weight as independent variables. The form of the metabolic (R) models, linearized by $\log_e$ transformation, was

$$[2] \quad R\ (\text{g O}_2\cdot\text{g}^{-1}\cdot\text{d}^{-1}) = \text{RA}\cdot W^{\text{RB}}\cdot e^{\text{RQ}\cdot T}$$

where RA is the intercept (g O_2·d^{-1}) for a 1-g fish at 0°C, RB is the exponent of the weight-dependence function, W is the wet weight (g) of the fish, RQ is the temperature-dependence exponent, and T is temperature. This model structure was initially selected because it is compatible with existing bioenergetics software (Hewett and Johnson 1992, but see the Appendix). However, subsequent regression analysis found this model form to provide the best fit to the data.

Routine metabolism results

The laboratory metabolic data fit least-squares models well. Regression coefficients of determination (r^2) ranged from 0.75 to 0.95 for all species and ages considered (Table 5). Intercepts (RA) for the models (which are the values we used in bioenergetics models) were lowest for age-0 weakfish (0.0009) and age-0 striped bass (0.001 46) and highest for bluefish (0.0056). Age-0 striped bass and bluefish had the strongest mean size dependence of metabolism with allometric exponents (RB) of −0.27 and −0.264, respectively. Weakfish exhibited the least size dependence of all species (RB = −0.125, −0.155), but age-0 weakfish had the highest mean temperature dependence with RQ = 0.091, which was significantly higher than for age-0 striped bass (0.0834), bluefish (0.0693), or age-1 and older weakfish. Older weakfish had the smallest mean temperature-dependent exponent (0.0508) of all models (Table 5).

The allometric exponent of each species or age group was used to standardize laboratory metabolism data to 30, 100, 350, and 1000 g for comparison of temperature effects across species and sizes. Metabolism of all species increased with temperature throughout the range of temperatures examined (Fig. 3). Bluefish metabolism increased approximately fourfold between 15 and 29°C. Weakfish metabolism increased only twofold for a 30-g fish between 13 and 25°C, and less than twofold for larger fish between 15 and 29°C. Striped bass metabolism increased nearly threefold for a 30-g fish between 15 and 30°C, but increased only about twofold for a 1000-g fish at similar temperature ranges (Fig. 3).

Analysis of covariance among predators for routine metabolism of each size of fish (30, 100, 350, and 1000 g) showed the metabolism of weakfish and striped bass to be similar. Slopes for the temperature effect on metabolism were not significantly different ($P > 0.05$) for all sizes of striped bass and weakfish. Further analysis showed no significant differences (ANCOVA, $P > 0.05$) in metabolism

for the 30- and 1000-g striped bass and weakfish. Routine metabolism data for bluefish were significantly different from those of weakfish and striped bass in all comparisons except for the data for 100-g striped bass metabolism, which had a similar slope but different intercept from that of the bluefish.

Bluefish, which had the highest C_{max} rates, also had the highest routine metabolic rates of all species and sizes. Striped bass and weakfish had similar specific metabolic rates at each temperature and fish size (Fig. 3).

Bioenergetics model development

The bioenergetics models for striped bass, bluefish, and weakfish were based upon the balanced energy equation (eq. 1), (Winberg 1956; Kitchell et al. 1977). Earlier error and sensitivity analyses on bioenergetics models of this type found that models were very insensitive to errors in the parameters for egestion, excretion, and specific dynamic action (SDA) (Stewart et al. 1983; Bartell et al. 1986). On the basis of these studies, and because a review of the energy budget literature by Brett and Groves (1979) suggested these components to represent a small component of the energy budgets of carnivorous fish, we elected to model egestion (F) and excretion (U) and specific dynamic action (SDA) as constant proportions of ingested or assimilated energy as has previously been done (Kitchell et al. 1974, 1977; Rice et al. 1983; Bevelhimer et al. 1985; Moore 1988; Hewett and Johnson 1992). We assumed that egestion was a constant proportion (0.104) of consumption (C). Excretion (0.068) and SDA (0.172) were modeled as a constant proportion of assimilated energy ($C - F$). These proportions were used in each species model and are the same as those used by Moore (1988) to model energetics of striped bass in a Virginia reservoir.

The consumption and metabolism component of the energy equation (eq. 1) have temperature- and size-dependent functions. We defined size dependency of C_{max} on the basis of the allometric functions for C_{max} of each species (Tables 1–3). Temperature dependency of C_{max} was modeled with the Thornton and Lessem (1978) algorithm for size-specific data on C_{max}. The Thornton and Lessem (1978) algorithm has been reported to provide a better fit to C_{max} data as a function of temperature than other models (such as the Q_{10} relationship used by Kitchell et al. 1977; see Hewett and Johnson 1992). The Thornton and Lessem (1978) algorithm is the product of two sigmoidal curves with a range from 0 to 1. The product of the sigmoidal curves f(T) is multiplied by the C_{max} value for a fish of a given size fish at the observed temperature as determined by the allometric equation model. The complete C_{max} model is

$$[3] \quad C_{max} = CA \cdot W^{CB} \cdot f(T)$$

One of the sigmoidal curves fits temperature-dependency data as it ascends (Θ_1 to Θ_2) and the other fits the descending data (Θ_3 to Θ_4). Further detail on the Thornton and Lessem algorithm can be found in Hewett and Johnson (1992) and Thornton and Lessem (1978).

The temperature dependence of C_{max} appeared to change with ontogeny for fish in our study as was also suggested by Kitchell et al. (1974), Coutant et al. (1984), Stewart and Binkowski (1986), and Rudstam (1988). Therefore, we stratified the C_{max} data by size groups as for the C_{max} comparisons and fit curves to the observed laboratory data by iterative manual adjustments for each age group on the basis of these stratifications. We fitted the temperature-dependent model of Thornton and Lessem (1978) by eye for age-0, -1, and -2 and older bluefish and weakfish, and age-0, -1, -2, and -3 and older striped bass (Tables 1–3).

We modeled the metabolism of striped bass, bluefish, and weakfish with the temperature- and weight-dependent models described in Table 5. The activity multiplier (ACT) is a component of bioenergetics models that is used to adjust resting or routine metabolism to active metabolism produced

Fig. 1. Comparison of laboratory-derived size dependency of maximum consumption rates (C_{max}) for striped bass (A), bluefish (B), and weakfish (C) at optimal temperatures (20–25°C) for consumption.

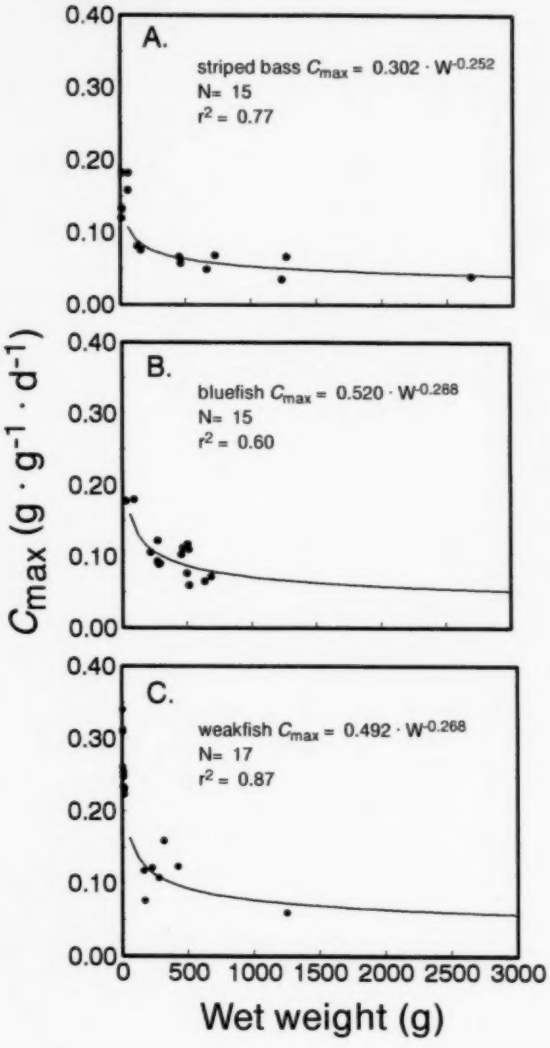
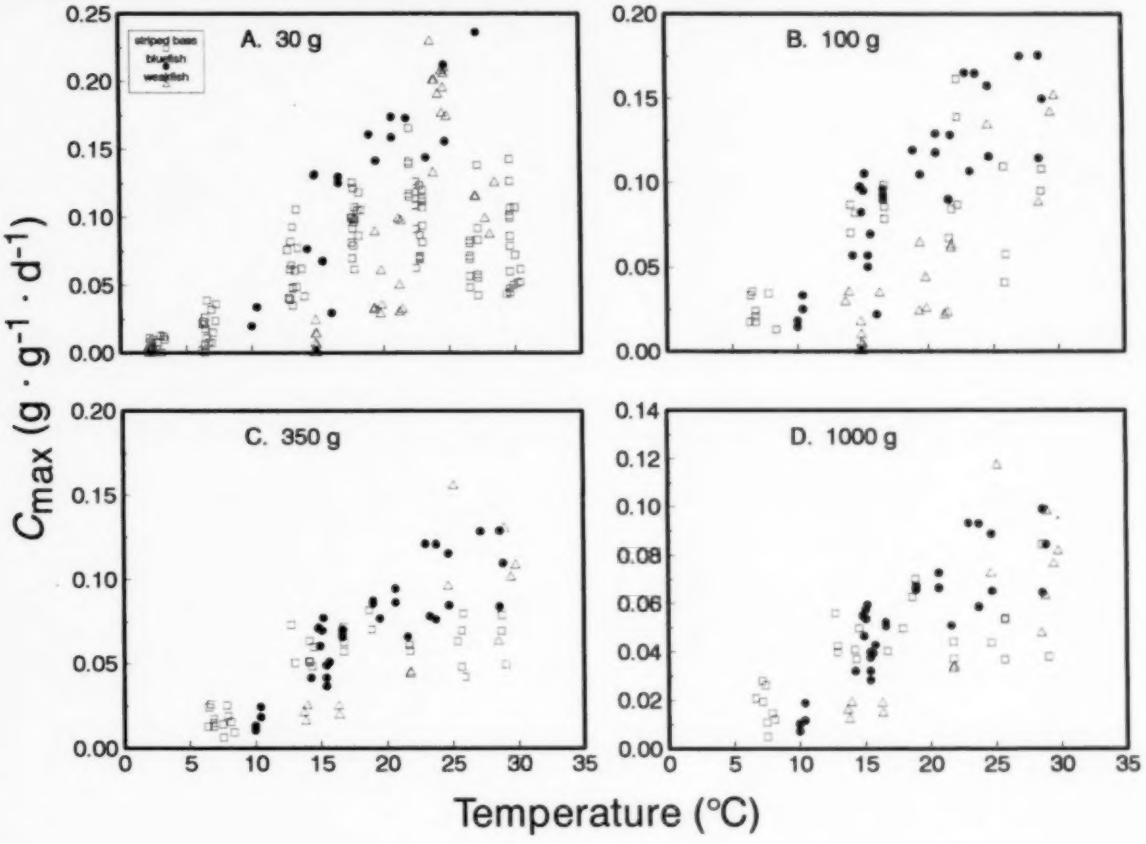


Fig. 2. Comparison of temperature dependency of C_{max} for striped bass, bluefish, and weakfish. Laboratory data were standardized to a 30-g fish (A), 100-g fish (B), 350-g fish (C), and 1000-g fish (D).



during normal daily activity such as feeding, swimming, and diel migrations. (Winberg 1956; Brett and Groves 1979). The complete metabolism equation is described by

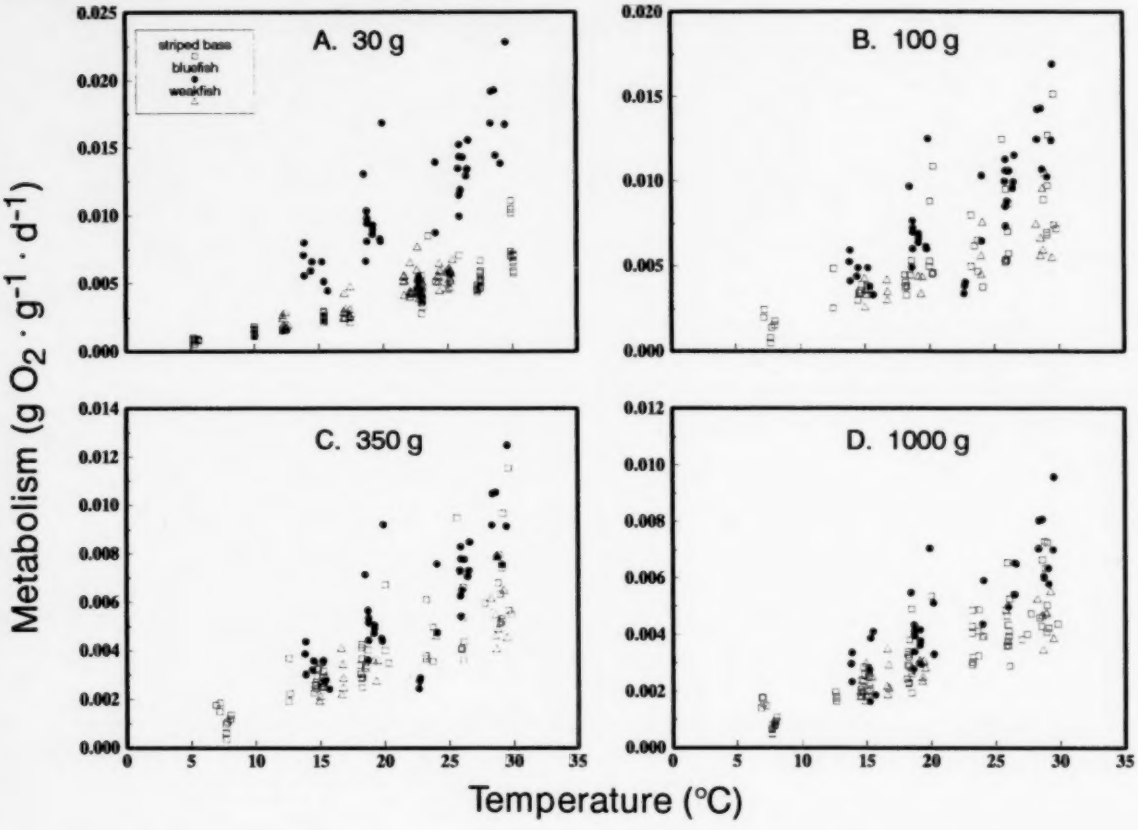
$$[4] \quad R = \mathrm{RA} \cdot W^{\mathrm{RB}} \cdot e^{\mathrm{RQ} \cdot \mathrm{ACT}}$$

where all parameters are as in eq. 2, and eq. 2 is multiplied by ACT to estimate total metabolism (R, g $O_2 \cdot d^{-1}$). If we assume an oxycalorific value of 3240 cal·g^{-1} (1 cal = 4.184 J), which was derived from Elliott and Davison (1975), oxygen consumption intercepts (RA) can be converted to energy units.

We estimated ACT for the age-0 striped bass, age-0 weakfish, age ≥ 1 striped bass, age ≥ 1 weakfish, and bluefish models indirectly from the maximum consumption experiments for each species. Because of low growth rates and a lack of estimates of energy density for fish in experiments at low temperatures, only data from experiments at temperatures above 14°C for weakfish, above 15°C for bluefish, and above 18°C for striped bass were used for the estimates of ACT. We set up each bioenergetics model in a spreadsheet to estimate ACT. We used observed measures of growth, consumption, temperature, and energy density of predators and prey as inputs to the bioenergetics model spreadsheet. We then solved for the value of ACT needed to balance the bioenergetics models so that predicted and observed growth (average daily energy gain) were equal. Data on the growth, temperature, and consumption came directly from the C_{max} experiments. Energy density of predators and prey was estimated from dry weight to wet weight percentages determined from fish used in experiments to estimate ACT. We killed predators at the end of these experiments. Predators and subsamples of prey were then dried to a constant weight at 70°C and energy density estimates were obtained with species-specific regressions of energy density on percent water content (Hartman and Brandt 1995*b*). The indirect measures of ACT were then averaged to arrive at the value of ACT used in the bioenergetics models (Tables 1–3). The estimates of ACT were based upon means of 14 observations for age-0 striped bass, 17 observations for age ≥ 1 striped bass, 9 observations for bluefish, 30 observations for age-0 weakfish, and 14 observations for age ≥ 1 weakfish.

Initial examination of data from the estimates of ACT showed a relationship between specific consumption (g·g^{-1}·d^{-1}) and ACT for each model. The relationship between specific consumption and ACT was linear in all

Fig. 3. Comparison of the effect of temperature on laboratory measures of routine metabolism of striped bass, bluefish, and weakfish. Laboratory data were standardized to a 30-g fish (A), 100-g fish (B), 350-g fish (C), and 1000-g fish (D) for comparison.



cases. The specific consumption rate explained 49% of the variation in ACT for bluefish and 50% of the variation in ACT for the age-1 and older striped bass model. For age-0 weakfish, age-1 and older weakfish, and age-0 striped bass models, the consumption rate explained 79–85% of the variation in ACT (Table 6).

Estimates of the mean ACT for each bioenergetics model were required for applications using the Hewett and Johnson (1992) bioenergetics software. Estimates of mean ACT varied with the species or cohort modeled. ACT ranged from 1.65 for age-1 and older striped bass to 3.43 for age-0 weakfish (Tables 1–3).

Model validation

We conducted independent growth experiments to validate the accuracy of the bioenergetics models for striped bass, bluefish, and weakfish. We ran the growth experiments at two temperatures for age-0 striped bass (6 and 20°C) and at one temperature for older striped bass (19.1°C), age-0 weakfish (24.7°C), older weakfish (25.1°C), and bluefish (23.5°C). Two temperatures were used in growth experiments for age-0 striped bass to validate the models at a range of temperatures. Low growth and small numbers of weakfish and bluefish prevented the evaluation of the bioenergetics models at low temperatures for bluefish, weakfish, and older striped bass. Fish were used only once in model validations. Model validations were replicated 14 times (individual fish) at each temperature for age-0 striped bass, 11 times for age ≥ 1 striped bass; 4 times for bluefish; 5 times for age-0 weakfish; and 2 times for age ≥ 1 weakfish (Table 7). We compared the consumption and growth rates measured during the experiment with the consumption rate (from the growth and temperature data) and growth rate (from the ration and temperature data) predicted by the bioenergetics model. All validations were completed using a spreadsheet in which the bioenergetics models were coded. Validations were made for both the constant ACT (consumption and growth) and the variable ACT predicted from regressions involving consumption rate (Table 6). For the variable ACT, only growth validations were possible because consumption validations would require solving for two unknown quantities simultaneously in the equations.

General procedures for model validation experiments were similar to those in C_{max} experiments. Individual fish

Table 5. Least-squares regressions predicting metabolism of striped bass, bluefish, and weakfish (g $O_2 \cdot g^{-1} \cdot d^{-1}$) as a function of fish wet weight (W) and temperature (T).

Model	N	r^2	RA	RB	RQ
Age-0 striped bass	86	0.95	0.001 456	−0.270	0.083
Age ≥ 1 striped bass	112	0.80	0.002 800	−0.218	0.076
Age-0 weakfish	50	0.95	0.000 858	−0.125	0.091
Age ≥1 weakfish	27	0.77	0.002 981	−0.155	0.051
Bluefish	58	0.75	0.005 580	−0.264	0.069

Note: The form of the metabolism (respiration) models was R (g $O_2 \cdot g^{-1} \cdot d^{-1}$) = $RA \cdot W^{RB} \cdot e^{(RQ \cdot T)}$, where RA is the exponential of the intercept for a 1-g fish at 0°C, RB is the exponent of the weight-dependence function, RQ is the temperature-dependence exponent and T is temperature. The 95% confidence limits on model variables are given in Tables 1–3.

Table 6. Least-squares regressions predicting ACT from specific consumption rate ($g \cdot g^{-1} \cdot d^{-1}$) for striped bass, bluefish, and weakfish bioenergetics models.

Model	N	r^2	a	b	F	P
Age-0 striped bass	14	0.85	0.356	26.987	66.2	0.0001
Age ≥ 1 striped bass	17	0.50	0.810	14.973	14.8	0.0016
Age-0 weakfish	30	0.79	2.074	16.677	103.26	0.0001
Age ≥ 1 weakfish	14	0.84	0.832	24.706	64.1	0.0001
Bluefish	9	0.49	0.449	27.416	6.7	0.0361

Note: The form of the model is: ACT = $a + b(C)$, where a and b are empirical constants and C is the specific consumption rate.

(age 0) or groups of two or three fish (age > 0) were handled and acclimated as for the maximum consumption rate experiments. Fish were fasted for 24 h prior to weighing, then fish were introduced into each experimental tank. Tank sizes for different sized predators were the same as those used for the C_{max} experiments. Fish were fed a known ration (across tanks 0.010–0.082% body weight/d for age-0 striped bass, 0.019–0.063% body weight/d for age ≥ 1 striped bass, 0.032–0.055% body weight/d for bluefish, 0.045–0.91% body weight/d for age-0 weakfish, and 0.067–0.076% body weight/d for age ≥ 1 weakfish) daily and temperatures recorded twice daily.

Fish almost always consumed all of the food offered because these ration levels were below the size-specific C_{max} levels observed. If food was left uneaten, it was removed from the tank after 1 h and weighed. At the end of the growth experiments, fish were fasted for 24 h, weighed to determine growth during the experiment, sacrificed by overdosing in MS-222, and dried to a constant weight in a 70°C oven. Dry weight as a percentage of wet weight was used to estimate energy density of the predator from species-specific energy density models (Hartman 1993; Hartman and Brandt 1995*b*). Energy densities of prey used in the experiments (thawed bay anchovy for striped bass and bluefish, live *Fundulus* spp. for weakfish) were estimated from an energy density regression measured for bay anchovy and a generic (all species combined) energy model for *Fundulus* spp. (Hartman 1993; Hartman and Brandt 1995*b*).

Validations with mean ACT

Overall, model estimates of consumption based on growth using mean ACT were closer to observed values than model predictions of growth based on observed consumption, and age-0 striped bass model predictions were much closer to measured values at 20.2 than at 6.9°C (Table 7). Because growth and consumption were near zero and day-to-day variability in consumption rates for individual fish was high in the 6.9°C validation experiment with striped bass we feel these results may be unrealistic. Thus, the actual ACT may often have been closer to 1.0 for these fish, and the model using mean ACT would tend to underestimate growth and overestimate consumption.

Excluding the cold water age-0 striped bass validations, predicted consumption averaged across all replicates was within −1.4 to +4.5% of observed consumption for each model with mean ACT. In contrast, growth estimates using mean ACT (averaged across all replicates) was predicted only within −7.1 to +30.1% of observed values (Table 7). The age-0 striped bass model overestimated consumption and underestimated growth at both test temperatures (Table 7). Agreement between observed and predicted values for striped bass was closest at 20°C where predicted consumption (averaged across replicates) was within +1.8 ± 7.7% (mean ± SD) and predicted growth was within −6.1 ± 26.2% of observed values. At 6.9°C, agreement was poorer, with growth underestimated by 24.2 ± 149.2% and consumption overestimated by 33.0 ± 22.2%. A possible explanation for the poorer agreement at 6.9°C was that growth

Table 7. Comparison of growth (using consumption data) and consumption (using growth data) for constant ACT and with ACT as a variable of consumption as estimated by the species and cohort-specific bioenergetics models (age-0 striped bass, age-1 striped bass, bluefish, age-0 weakfish, and age ≥ 1 weakfish) as a percentage of the observed values from independent laboratory validation experiments for each model.

	No. of experiments	Constant ACT		Variable ACT (Growth prediction, %)
		Growth prediction, (%)	Consumption prediction, (%)	
Age-0 striped bass model				
6.9°C	14	−24.2 (−110.3, 62.0)	+33.0 (20.1, 45.8)	+108.0 (55.3, 160.7)
20.2°C	14	−6.1 (−21.2, 9.1)	+1.8 (−2.6, 6.2)	+5.9 (−6.1, 17.9)
Age ≥ 1 striped bass model				
19.1°C	11	+21.0 (−5.4, 47.4)	−1.4 (−4.3, 1.5)	+75.6 (−15.9, 167.1)
Bluefish model				
23.5°C	4	+30.1 (−62.2, 122.4)	+1.1 (0.3, 1.9)	+428.8 (−365.6, 1223.2)
Age-0 weakfish model				
24.7°C	5	−29.1 (−76.2, −18.0)	+4.5 (−2.0, 11.0)	−103.8 (−198.2, −9.5)
Age ≥ 1 weakfish				
25.1°C	2	−7.1 (−41.2, 27.1)	+2.3 (−11.2, 15.8)	−103.8 (−94.9, 69.4)

Note: Comparisons for each bioenergetics model are given as means and 95% confidence invervals are in parentheses, for all replicates at each temperature.

during the experiment was very low (averaging ≤0.5 g/fish over 12 days). Because the models are very sensitive to growth estimates (Kitchell et al. 1977; Bartell et al. 1986) and because the measures of wet fish weights are relatively imprecise, a marginal weight change of more than 0.5 g is probably necessary to accurately validate the models.

The model for age ≥ 1 striped bass slightly underestimated consumption while overestimating growth. For the 11 validation replicates for age ≥ 1 striped bass (all at about 19°C), consumption predictions averaged −1.4%, but individual replicates ranged from −8.85 to +6.19% of observed values. Predictions of growth were more variable in their ability to match observed values. Bioenergetics model predictions overestimated growth by an average of +21.0% across all replicates (Table 7).

The bioenergetics model for bluefish and age 0 and age ≥ 1 weakfish was better at predicting consumption from growth than predicting growth from consumption data. Consumption was slightly overestimated (+1.1 to +4.5%) in each of these models and growth was overestimated in the bluefish model and underestimated in the weakfish models (Table 6).

Validations with variable ACT

Comparison of bioenergetics model predictions of growth (from consumption) for models with the variable ACT (ACT varies as a function of consumption rate) provided poorer fits to the validation data than did mean ACT models (Table 7). The best results were for striped bass and the poorest results for bluefish. The variable ACT model for age-0 striped bass provided growth estimates within 5.9% of observed values at 20°C, but was only within 108.0% at 6.9°C. The age-1 and older striped bass model with variable ACT overestimated growth by 75.6% compared with +21% for the mean ACT validations (Table 7). The variable ACT models for age-0 and age-1 and older weakfish overestimated growth by 103.8% compared with underestimates of −7.1 to −29.1% for age ≥ 1 and age-0 mean ACT models, respectively. Variable ACT models for bluefish overestimated growth by 428.8%, exceeding the mean ACT prediction by about 400% (Table 7).

Scope for growth

We used the bioenergetics models to construct scopes for growth (Kitchell et al. 1977) for predators of similar size for each species. Scopes for growth illustrate how overall energy budgets change with temperature for fish feeding at maximal levels (Kitchell et al. 1977). We compared energy budgets for two sizes of fish, 30 and 1000 g, as representatives of fish sizes corresponding to age-0 fish and adults.

Scope for growth differed considerably both among species and among fish sizes within species (Figs. 4, 5). For the small (30 g) fishes the optimal temperatures for growth (greatest margin between total costs: $F + U + \text{SDA} + R$, and C_{max}) were at 18.5°C for striped bass, 20°C for bluefish, and 23.5°C for weakfish (Fig. 4). Positive growth

Fig. 4. Scope for growth of 30-g bluefish, striped bass, and weakfish feeding at C_{max} as derived from the bioenergetics models for each species. *C*, C_{max}; *M*, active metabolism; *S*, specific dynamic action + *M*; *F*, egestion + *S*; *U*, excretion + *F*.

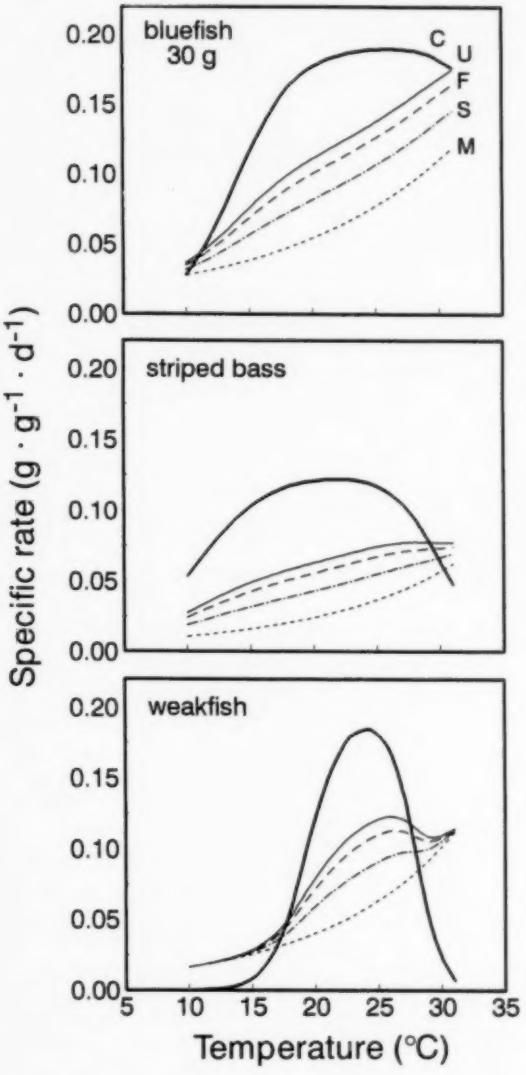
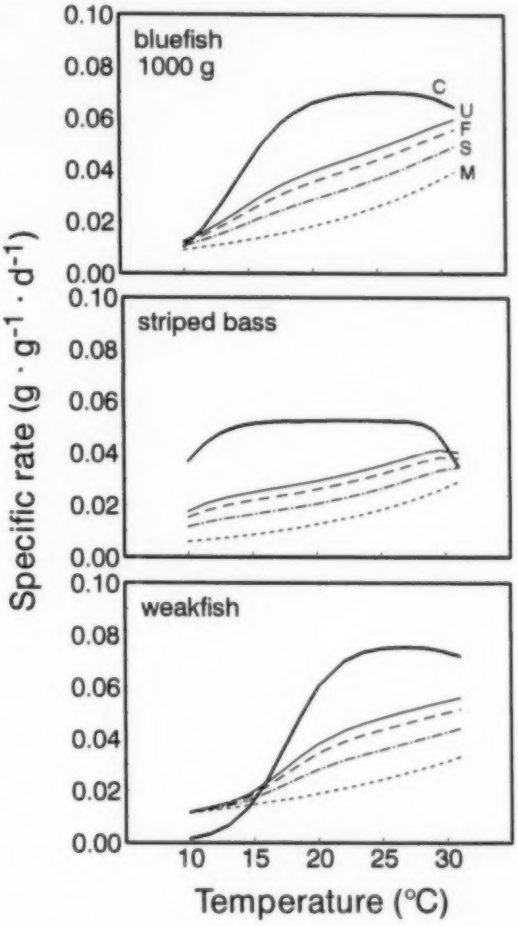


Fig. 5. Scope for growth of 1000-g bluefish, striped bass, and weakfish feeding at C_{max} as derived from the bioenergetics models for each species. *C*, C_{max}; *M*, active metabolism; *S*, specific dynamic action + M; *F*, egestion + S; *U*, excretion + *F*.



was possible for small striped bass from about 10 to about 29°C and for small bluefish from 11 through 30°C. Small weakfish had the smallest range of temperatures for which positive growth potential exists, 17–27°C; however, they had the largest growth potential, 7.1%/d at 23.5°C compared with 6.7%/d (20°C) for bluefish and 5.9%/d (18.5°C) for striped bass (Fig. 4).

Scope for growth of 1000-g fish generally had a broader range of temperatures at which near maximal growth was possible than for small predators. For striped bass, the optimal temperature for growth was 15°C, but growth was possible between about 6 and 30°C. At 15°C, the striped bass growth potential was 2.7%/d, nearly identical to the growth potentials for bluefish and weakfish at their optimal temperatures of 20 and 23.5°C, respectively (Fig. 5). The striped bass growth potential was >2%/d from about 12 to 25°C, whereas growth potential met or exceeded 2%/d from 16 to 26°C for bluefish and from 20 to 29°C for weakfish (Fig. 5). Growth was possible between 11 and 30°C for bluefish and between 16 and 30°C for weakfish (Fig. 5).

Discussion

Comparisons of scope for growth among 1000-g striped bass, bluefish, and weakfish revealed an interesting trend toward reduced thermal influence on the growth potential of larger, older fish. Previous bioenergetics modeling work

with esocids, salmonids, and percids was based on laboratory measurements of the temperature dependency of consumption using small fish (Kitchell et al. 1977; Stewart et al. 1981, 1983; Bevelhimer et al. 1985; Wahl and Stein 1991). We used a wide range of fish sizes for C_{max} and metabolic measurements for each species. Our laboratory-validated bioenergetics models indicated that larger fish were capable of achieving near maximal growth rates over a wider range of temperatures than has been assumed or reported for other species. The temperature for maximal growth in striped bass declined with size of fish from 18.5°C at 30 g to 15°C at 1000 g, which is consistent with previous studies for walleye (*Stizostedion vitreum*) (Kitchell et al. 1977), esocids (Wahl and Stein 1991), alewife (*Alosa pseudoharengus*) (Stewart and Binkowski 1986), and salmon (Beauchamp et al. 1989). However, no ontogenetic differences in thermal optima for growth were found for weakfish and bluefish weighing between 30 and 1000 g.

The laboratory data for C_{max} of striped bass, bluefish, and weakfish clearly showed an ontogenetic change in the shape of the temperature-dependency curves within a species. Larger fish had a broader range of temperatures at which near maximal C_{max} values were achieved than did younger and smaller fish. C_{max} of larger fish also did not decline as rapidly at upper temperatures as it did for small fish. Previous bioenergetics models have typically used the same temperature-dependency functions of C_{max} for juvenile through adult fishes, with these functions simply being shifted so that the optimal temperature of consumption (where C_{max} is highest) occurred at lower temperatures for larger fish (Kitchell et al. 1974, 1977; Stewart and Binkowski 1986; Moore 1988; Rudstam 1988). Our data for striped bass, bluefish, and weakfish suggest that important ontogenetic differences in the shape of the temperature dependence of C_{max} occur for these species and that C_{max} models derived only from juvenile fish may fail to accurately reflect the consumption potential of larger fish.

The energetics of striped bass suggest that they are a more generalist species, adapted to a wide range of environmental conditions. Scope for growth of striped bass was near optimal under a broader range of environmental temperatures than that of bluefish or weakfish. This wide thermal adaptation was in part due to the broad range of temperatures at which peak maximum consumption was achieved, and in part to the low metabolic rates for striped bass relative to bluefish or weakfish. Although bluefish and weakfish did not appear to be adapted to as wide a range of temperatures as striped bass, the C_{max} values at typical temperatures during estuarine residency by weakfish and bluefish (temperatures > 17°C) were considerably higher than for similar-sized striped bass at the same temperature. The higher specific consumption rates by weakfish and bluefish mean that their potential predatory impact per individual may be greater than for striped bass, which have lower specific rates.

For comparing the energetics of striped bass, bluefish, and weakfish, scope for growth provides more integrated information than comparisons of laboratory measurements of either metabolism or C_{max}. Comparisons of size-specific metabolic rates of fish yield only information on the dynamics of oxygen consumption of fasting fish under restricted conditions. By constructing bioenergetics models for these species and indirectly deriving the activity multiplier of metabolism, ACT, we were able to more adequately compare the energetics of striped bass, bluefish, and weakfish. Laboratory data suggest that bluefish consistently had higher specific metabolic rates than striped bass or weakfish across temperatures and fish sizes. However, total metabolic costs of weakfish feeding at C_{max} were roughly equivalent to those of similar-sized bluefish at the same temperatures.

In bioenergetics models of the type we describe, the ACT is usually assumed to be a constant value between 1 and 2 on the basis of the relative activity believed to be experienced by the fish (Kitchell et al. 1977; Rice et al. 1983; Hewett and Johnson 1992). Brett and Groves (1979) suggested that active metabolism was 2–3 times the basal metabolic rates for most fish. We solved for ACT in our models by choosing the ACT required to balance the energy equation using data from the consumption experiments. Estimates of ACT ranged from 1.65 to 3.43 for the striped bass, bluefish, and weakfish models. These estimates of ACT are on the higher end of values used in most previous bioenergetics modeling applications, but are similar to those reported for indirect measures of ACT for field populations of yellow perch (*Perca flavescens*) (Boisclair and Leggett 1989), young walleye (Madon and Culver 1993), and young brook charr (*Salvelinus fontinalis*) (Boisclair and Sirois 1993) and are similar to the values of 2–3 suggested by Brett and Groves (1979).

We found that our indirect estimates of ACT were positively related to specific consumption rate for each species. In experiments to estimate ACT, specific consumption rate ($g \cdot g^{-1} \cdot d^{-1}$) explained 49–85% of the variation in ACT. Other studies have found that ACT increased with specific consumption rate for walleye (Madon and Culver 1993) and brook trout (*Salvelinus fontinalis*) (Boisclair 1992). These findings that ACT varies as a positive function of consumption rate are problematic for typical applications of bioenergetics models. Bioenergetics models are commonly used to estimate consumption from field estimates of growth, diet, energy content, and temperatures (Ney 1990; Hartman and Margraf 1992; Hewett and Johnson 1992). Because the energy budget must balance (energy ingested must equal growth plus losses), eq. 1 can easily be solved for consumption when the other variables are known or estimated. However, if ACT varies as a function of C the energy losses now have an unknown variable and solving the equation for C requires simultaneous solution for two unknown quantities. In laboratory validation experiments for each bioenergetics model we found that use of a constant ACT resulted in good estimates of consumption and somewhat poorer estimates of growth. However, incorporation of the variable estimate of ACT (as a function of C) resulted in poorer estimates of growth in the validation experiments. The poor performance of the variable ACT models in the validation experiments was surprising because regression analysis found consumption rate to explain 49–85% of the variability in estimates of ACT. We must conclude that further study of the complex interaction between feeding factors (e.g., temperature, fish size, proportion of maximum consumption) and activity multiplier is needed. However,

because of the positive results of model validations with mean ACT (and the poor results with variable ACT) and owing to the complexities of simultaneously solving for two unknowns in an equation, we recommend that for the models presented here, mean ACT should be used for estimating consumption and growth.

Energetic rates measured for Chesapeake Bay piscivores were similar to those reported for other fishes. Intercept values (CA) for the C_{max} functions (1-g fish at optimal temperature) ranged from 0.302 for striped bass to 0.520 for bluefish. Intercepts reported for freshwater species range from 0.182 for bluegill (*Lepomis macrochirus*) (Kitchell et al. 1974) to 1.57 for a generalized corregonid (Hewett and Johnson 1992); however, consumption intercepts for piscivores have typically had a narrower range, from 0.20 to 0.33 (Rice et al. 1983; Moore 1988; Beauchamp et al. 1989; Stewart and Ibarra 1991). Size dependency of C_{max} exponents for fish modeled in this study were not significantly different from each other, but ranged from −0.25 (striped bass) to −0.29 (bluefish). The size dependency exponents in other studies normally range from −0.18 to −0.35 for piscivores (Hewett and Johnson 1992).

Measured metabolic rates and models developed from these measurements were also somewhat similar to other reported values, but comparisons were complicated by the structure of the metabolism model. We used a new approach to describe metabolism of striped bass, bluefish, and weakfish that is based on aspects of both the Stewart et al. (1983) model and the Kitchell et al. (1977) model. The Stewart et al. (1983) model describes metabolic rates as an exponential function of temperature with activity varying as a function of swimming speed. Swimming speed is also a function of temperature and fish size. The Kitchell et al. (1977) model is a nonlinear function based on three parameters that uses a Q_{10} relationship to model metabolic rate as a function of temperature with a constant activity multiplier value to correct standard metabolism for normal activity. Because our metabolism data increased exponentially with temperature through about 30°C, we used the model of Stewart et al. (1983) to describe metabolism. However, in the absence of any swimming speed data to correlate with the metabolism data, we used a constant activity multiplier (ACT) as did Kitchell et al. (1977) to estimate the metabolism of actively swimming fishes.

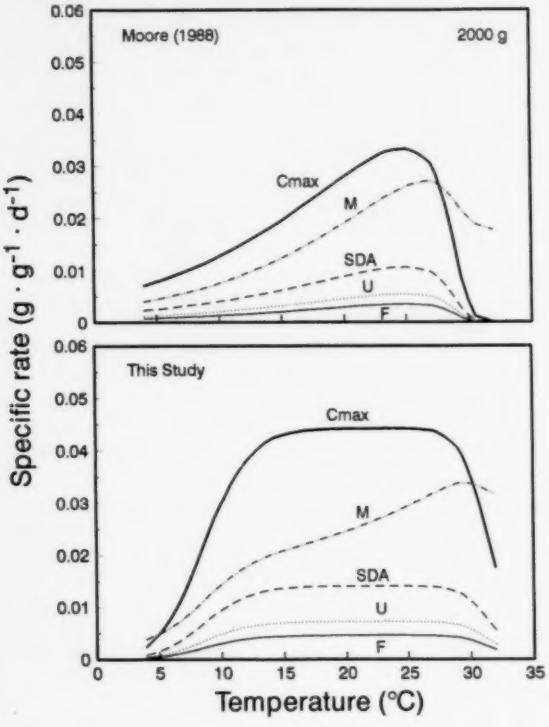
The intercept values of the metabolism models represent the theoretical metabolic rate of a 1-g fish at 0°C. Metabolic rate intercepts (RA) in this study ranged from 0.0009 for age-0 weakfish to 0.0056 for bluefish. The metabolism intercept for the age-0 weakfish model is slightly lower than reported for other species, but is still close to the intercept for sockeye salmon (*Oncorhynchus nerka*) of 0.0014 (Beauchamp et al. 1989). The intercept for bluefish is only about 20% higher than the highest intercept in the literature, that for the more sedentary lake trout (*Salvelinus namaycush*) (Stewart et al. 1983). The size-dependency exponents for the metabolism models (RB) were also within the range reported for other species. Exponents in the striped bass, bluefish, and weakfish models ranged from −0.125 to −0.270, well within the range reported between corregonids (−0.12) and largemouth bass (−0.355) (Rice et al. 1983; Hewett and Johnson 1992).

Examination of the metabolism models for age-0 and age-1 and older striped bass suggests that the two curves do not intersect exactly. The fact that these two models developed from experiments in two different metabolic chambers do not connect exactly is not surprising. The intercepts for the age-0 metabolism model (0.001 456) and the age ≥ 1 metabolism model (0.002 80) differ by 48%. However, ACT must be considered in comparisons of the equations because respiration measures were routine rates and nominal activity levels likely differed between the life stages and chambers. If the intercept (RA, 1-g fish at 0°C) is multiplied by the values of ACT, the model intercepts for active metabolism become 0.0036 (age 0) and 0.0046 (age ≥ 1), a difference of only 22% and not significantly different given the large 95% confidence intervals about RA for age-0 striped bass. Similar comparison of the metabolism models for age-0 and age ≥ 1 weakfish shows a larger difference between the RA values of age-0 (0.0009) and age ≥ 1 (0.0030) models. Factoring in ACT to examine active metabolism yields intercepts of 0.003 09 for age 0 and 0.0074 for age ≥ 1 weakfish. Consideration of the 95% confidence intervals for intercepts and ACT in the weakfish metabolism model comparisons yields values of 0.0036 (age 0) and 0.004 36 (age ≥ 1), only a 17% difference.

A great deal of previous energetics work has been done on striped bass. However, none had the range of fish sizes and temperatures used in this study. Klyashtorin and Yarzhombek (1975) studied metabolism of striped bass ranging in size from 0.3 to 22 g at 22°C. Kruger and Brocksen (1978) measured the metabolism of fish ranging in size from 22 to 68 g at 8–24°C. Measures of growth and consumption of striped bass by Cox and Coutant (1981) were conducted on fish averaging 96–294 g at 11–34.5°C. In this study we measured the metabolism and maximum consumption of striped bass ranging in size from 5 to 3100 g at temperatures ranging from 2 to 30°C. Not only do the data derived from this study provide better coverage of fish-size effects than was previously reported, but the temperature effects are also more complete, particularly at higher temperatures. Comparison of the metabolism estimates from these other studies with those found here for striped bass shows them to be similar. For a 1-g fish at 22°C Klyashtorin and Yarzhombek (1975) measured oxygen consumption rates of 0.013 to 0.017 g $O_2 \cdot d^{-1}$. Similarly, the data of Kruger and Brocksen (1978) indicate that a similar-size fish at the same temperature would consume 0.011 g $O_2 \cdot g^{-1} \cdot d^{-1}$. These rates are very similar to those estimated in this study. Rates are slightly different for each striped bass model (age 0 or age ≥ 1), but range from 0.009–0.022 g $O_2 \cdot d^{-1}$ for resting and active 1-g fish at 22°C in the age-0 model, to 0.005–0.008 g $O_2 \cdot g^{-1} \cdot d^{-1}$ for resting and active fish in the model for age ≥ 1 striped bass.

Moore (1988) developed a bioenergetics model for juvenile and adult striped bass for use in Virginia reservoirs. Moore (1988) and Moore et al. (1993) used the data of Cox and Coutant (1981) and Kruger and Brocksen (1978) to estimate parameters for the bioenergetics model. Our bioenergetics models for striped bass differ significantly from that constructed by Moore (1988). The Moore (1988) model used the equation of Kitchell et al. (1977) for the temperature dependence of consumption. We chose to use

Fig. 6. Comparison of the scope for growth for a 2000-g striped bass for the Moore (1988) model (above) and the striped bass model developed in this study (below). All rates are specific ($g \cdot g^{-1} \cdot d^{-1}$). Energy budget components are maximum consumption (C_{max}), active metabolism (M), specific dynamic action (SDA), excretion (U), and egestion (F).



the Thornton and Lessem (1978) algorithm because it provided a better fit to the experimental C_{max} data than the other models.

The Moore (1988) model differed from the ones developed here for striped bass for temperatures of C_{max}. We found the temperatures of C_{max} to be 22, 19, 18, and 15°C for age-0, age-1, age-2, and age-3 and older striped bass. These temperatures were 3.0, 3.0, 2.0, and 5.0°C lower than those used by Moore (1988) for age-0, age-1, and older striped bass.

Metabolism models also differed between the two studies. We found that the routine metabolism of age-0 and age-1 and older striped bass increased exponentially with temperature from 6 through 30°C and therefore modeled metabolism as an exponential function of temperature (Stewart et al. 1983; Stewart and Binkowski 1986). The Moore (1988) model used the temperature-dependence function of Kitchell et al. (1977) to model metabolism. The result is that for temperatures above 30°C, metabolic costs in the Moore (1988) model declined, while metabolic costs increased slowly above 30°C in our model. In both the Moore (1988) model and our models the metabolic rate was multiplied by a value (ACT) to represent the additional costs of activity over basal metabolism. Moore (1988) assumed that striped bass were fairly active and set ACT equal to 2.0 in his model. We algebraically solved the balanced energy equation to estimate ACT. The derived ACT for age-0 striped bass was about 25% higher and the derived ACT for age ≥ 1 striped bass about 18% lower than the Moore (1988) ACT, although the 95% confidence intervals for age-0 striped bass do encompass Moore's assumption of ACT = 2.0.

The Moore (1988) model predicts lower specific growth rates for all fish sizes and temperatures than do our models. For age-0 striped bass the Moore (1988) model predicts higher specific metabolism and lower potential specific growth rates from 7 to 30°C than the age-0 model. For a 2000-g striped bass, the metabolism costs are similar through 25°C, but metabolic costs decline in the Moore model, while they continue to increase slowly in our model (Fig. 6). Our models and laboratory data demonstrate higher possible maximum consumption than the Moore model.

The net result of the basic differences between the models of Moore (1988) and the present study is that striped bass have a larger scope for growth in our models than in Moore's (1988) model and that the upper temperature that supports positive growth is higher (30 vs. 28°C) in these models than in the Moore model (Fig. 6). As a consequence of the higher total metabolic costs for small (<100 g) striped bass in the Moore (1988) model, field estimates of consumption from growth data would be higher than those predicted with our models. Potential scope for growth was always higher in our models than would be possible in the Moore (1988) model, primarily due to differences in the temperature relationship of maximum consumption between the two models.

Comparisons of data from bluefish and weakfish with the previous studies are difficult because little work on the energetics of these species has been published. The only work related to bluefish is from a study of comparative swimming energetics in striped bass and bluefish (Freadman 1978). The bluefish study had a sample size of one, only at 15°C, with swimming velocities of 20–100 $cm \cdot s^{-1}$ (Freadman 1978) and thus was of little comparative value. Similar studies involving weakfish have not been published. However, an ad libitum feeding study with young weakfish from Delaware Bay is currently underway at the University of Delaware (T. Lankford, University of Delaware, Lewes, Del., personal communication) that should provide a basis for comparison with the C_{max} values derived in this study.

Bioenergetics models represent a significant improvement over simple energy budgets. Energy budgets typically present an accounting of the fate of energy within a particular species of fish. Usually such energy budgets are restricted to one temperature or one size of fish and thus do not provide as much detail as bioenergetics models regarding the effects of temperature and fish size on energy partitioning in fish.

For the limited sizes and temperatures described by fish energy budgets, the dynamics are similar to bioenergetics models. Tuncer et al. (1990) described the energy budgets of striped bass and striped bass hybrids (*M. chrysops* × *M. saxatilis*) under two feeding levels at one temperature

(23°C). At a ration level of 5% of body weight/d, striped bass were found to grow at about 1.3%/d or about 26% of ingested energy (Tuncer et al. 1990). Modification of the Tuncer et al. (1990) ration to account for the disparity in energy density between the striped bass (1367 cal·g^{-1}) and the commercial food (5162 cal·g^{-1}) resulted in a ration of about 0.189 g·g^{-1}·d^{-1} and growth of about 0.05 g·g^{-1}·d^{-1}. The Tuncer et al. (1990) budget is similar to that predicted by our age-0 striped bass model under similar conditions (ration of 0.189 g·g^{-1}·d^{-1}, temperature of 23°C, and fish size of 15 g wet weight). The age-0 striped bass model predicts that growth under these conditions would be 0.061 g·g^{-1}·d^{-1} or 32% of ingested energy. The two budgets differ by about 25% in terms of predicted growth. Metabolic costs have been reported to increase with fish density for striped bass (Hartman and Brandt 1993) and suggested for other species (Fox 1991). This would easily account for the disparity in growth between the Tuncer et al. (1990) energy budget and the current study. The energetics results for age-0 striped bass in this study are based on solitary fish, while those of Tuncer et al. (1990) were obtained on groups of 70 fish (1 fish/L). Striped bass usually school as juveniles. However, they probably do not achieve densities of 1 fish/L. Therefore, the actual proportion of energy allocated for growth might be intermediate to the values of this study and Tuncer et al. (1990), although the similarity in rates between the two studies suggests that the model provides reasonable measures of energy budgeting for striped bass.

The bioenergetics models for striped bass, bluefish, and weakfish should provide good estimates of consumption. Model validations using mean ACT generally found the bioenergetics models to be accurate at predicting consumption from growth data, but less accurate when predicting growth from consumption. The bioenergetics models generally predicted consumption within 10% of observed rates for averages across replicates, but predictions of growth were more variable. Predictions of growth with mean ACT averaged within −6% (20°C) to −24% (6.9°C) for age-0 striped bass, +21% for age ≥ 1 striped bass, +30% (23.5°C) for bluefish, −29% (24.7°C) for age-0 weakfish, and −7.1% (25.1°C) for age ≥ 1 weakfish. Similar predictive abilities and limitations have been reported for other bioenergetics models of the Wisconsin type (Minton and McLean 1982; Rice and Cochran 1984; Beauchamp et al. 1989; Wahl and Stein 1991). Beauchamp et al. (1989) found model estimates of consumption to agree with field-derived estimates within 10%. Similarly, Rice and Cochran (1984) found model estimates of consumption for largemouth bass to be within 8.5% of field estimates. However, sensitivity analyses of similar bioenergetics models have found such models to be very sensitive to changes in as many as seven model parameters when used to estimate growth (Bartell et al. 1986; Beauchamp et al. 1989). For this reason some have suggested using the models to predict consumption rather than growth (Kitchell et al. 1977; Beauchamp et al. 1989; Ney 1990).

Some caution must be used when applying the bioenergetics models for striped bass, bluefish, and weakfish to field situations. Although we attempted to validate the models in the laboratory, it is still an extrapolation to apply these models to the field. Such applications assume that the behavior and activity of the fish in laboratory tanks are similar to those in feral populations. The indirect estimates of ACT used in this study are an improvement over earlier assumptions regarding fish activity (Kitchell et al. 1977; Kitchell and Breck 1980; Rice et al. 1983; Shuter and Post 1990), but they are still extrapolations when applied to wild fish. Lucas et al. (1991) found field measurements of activity in northern pike (*Esox lucius*) using heart rate telemetry to be as much as 50% higher than assumed in previous bioenergetics models (Diana 1983). Direct measures of activity of wild striped bass, bluefish, and weakfish are complicated by their behavior and by ambient estuarine salinities that prevent the practical use of sonic tags to measure field metabolism (the signal is attenuated in seawater).

Bluefish are a highly active species and may have the greatest potential for poor extrapolation between laboratory and field activity. Although bluefish experiments were conducted in the largest available tanks, the size of these tanks may have constrained the activity of this fish to levels below those expected in the field. Observations of bluefish in tanks indicated that they were the most active of the three species, even at temperatures as low as 14°C; however, we cannot quantify how much activity may have been suppressed by the tanks. We did find from earlier manipulations of the bioenergetics models for all three species that ACT cannot be increased much above what was indirectly measured in the laboratory and still support field-observed growth without changing other model parameters such as C_{max}. Therefore, the bioenergetics models for striped bass, bluefish, and weakfish probably provide reasonable estimates of field consumption for these species.

Conclusions

The data and models generated in this study provide new insights into the energetics of estuarine predators and also point out potential problem areas in bioenergetics models. Modeling of complete energy budgets using bioenergetics models showed that the temperature effects on the metabolism and maximum consumption of large bluefish and weakfish are similar (and of higher specific rates than for striped bass), a fact that was not apparent from routine metabolism data. Further, we found that the shape of the temperature-dependency function changed with ontogeny, particularly for striped bass. The temperature-dependency function changed from one that increased sharply with temperature to a maximum and then declined rapidly at temperatures above optimum for young fish to a more gradually increasing function that appeared to be either asymptotic or to decline only slightly at higher temperatures for larger fish. This ontogenetic change in the temperature dependency of C_{max} points out the need to conduct experiments on large fish as well as juveniles to adequately model the consumption dynamics of large predators: juvenile consumption dynamics may not fit adults particularly well. Finally, the indirect measures of ACT were somewhat higher than those typically assumed in previous bioenergetics models. Although the actual values of ACT in the field may differ from those derived in the laboratory, field

measures of ACT are difficult to quantify. Recent advances in remote telemetry may make field measures of activity possible; however, they rely on some physiological correlate of metabolism such as cardiac rate, tail beating frequency, or measures of ventilatory rate to be effective and also are limited by tag size to relatively large individual fish (Lucas et al. 1991, 1993). Regardless of the uncertainties, the bioenergetics models developed here will have use as tools in the management and ecology of the Chesapeake Bay.

Acknowledgements

We thank the Potomac Electric Power Company's Chalk Point fish hatchery and the Maryland Department of Natural Resources Manning fish hatchery for providing us with fingerling striped bass for the study. We also thank H. Millsaps for offering help and suggestions and for maintaining the seawater system at Chesapeake Biological Laboratory during the study. We thank D. Hartman, T. Rippetoe, and A. Goyke for maintaining laboratory fish during several periods when the authors were away from the laboratory. Support for this study was provided by the Electric Power Research Institute's Fellowship Program in Population Biology (EPRI-09-07), the Sport Fishing Research Institute (SERP-90-21), the Maryland Sea Grant Program (NA90AA-D-SG063R/F-65), and the National Science Foundation's Biological Oceanography Program (OCE-9116071 and OCE-9415740).

References

Bartell, S.M., J.E. Breck, R.H. Gardner, and A.L. Brenkert. 1986. Individual parameter perturbation and error analysis of fish bioenergetics models. Can. J. Fish. Aquat. Sci. 43: 160–168.

Beamish, F.W.H. 1970. Oxygen consumption of largemouth bass, *Micropterus salmoides*, in relation to swimming speed and temperature. Can. J. Zool. 48: 1221–1228.

Beauchamp, D.A., D.J. Stewart, and G.L. Thomas. 1989. Corroboration of a bioenergetics model for sockeye salmon. Trans. Am. Fish. Soc. 118: 597–607.

Bevelhimer, M.S., R.A. Stein, and R.F. Carline. 1985. Assessing significance of physiological differences among three esocids with a bioenergetics model. Can. J. Fish. Aquat. Sci. 42: 57–69.

Boisclair, D. 1992. Relationship between feeding and activity rate for actively foraging juvenile brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 49: 2566–2573.

Boisclair, D., and W.C. Leggett. 1989. The importance of activity in bioenergetics models applied to actively foraging fishes. Can. J. Fish. Aquat. Sci. 46: 1859–1867.

Boisclair, D., and P. Sirois. 1993. Testing assumptions of fish bioenergetics models using direct estimates of growth, consumption, and activity rates. Trans. Am. Fish. Soc. 122: 784–796.

Brett, J.R. 1971. Satiation time, appetite, and maximum food intake of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 28: 409–415.

Brett, J.R., and T.D.D. Groves. 1979. Physiological energetics. *In* Fish physiology. Vol. 8. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York.

Carpenter, S.R., J.F. Kitchell, and J.R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience, 35: 634–639.

Carpenter, S.R., J.F. Kitchell, J.R. Hodgson, P.A. Cochran, J.J. Elser, M.M. Elser, D.M. Lodge, D. Kretchmer, X. He, and C.N. von Ende. 1987. Regulation of lake primary productivity by food web structure. Ecology, 68: 1863–1876.

Chao, L.N., and J.A. Musick. 1977. Life history, feeding habits, and functional morphology of juvenile sciaenid fishes in the York River estuary, Virginia. Fish. Bull. 75: 657–702.

Coutant, C.C., K.L. Zachman, D.K. Cox, and B.L. Pearman. 1984. Temperature selection by juvenile striped bass in laboratory and field. Trans. Am. Fish. Soc. 113: 666–671.

Cox, D.K., and C.C. Coutant. 1981. Growth dynamics of juvenile striped bass as functions of temperature and ration. Trans. Am. Fish. Soc. 110: 226–238.

Diana, J.S. 1983. An energy budget for northern pike (*Esox lucius*) in Lac Ste. Anne, Alberta. Can. J. Fish. Aquat. Sci. 37: 1454–1458.

Elliott, J.M. 1976. Energy losses in the waste products of brown trout (*Salmo trutta* L.). J. Anim. Ecol. 45: 561–580.

Elliott, J.M., and W. Davison. 1975. Energy equivalents of oxygen consumption in animal energetics. Oecologia, 19: 195–201.

Elliott, J.H., and L. Persson. 1978. The estimation of daily rates of food consumption for fish. J. Anim. Ecol. 47: 977–990.

Fox, M.G. 1991. Food consumption and bioenergetics of young-of-the-year walleye (*Stizostedion vitreum vitreum*): model predictions and population density effects. Can. J. Fish. Aquat. Sci. 48: 434–441.

Freadman, M.A. 1978. Swimming energetics of striped bass and bluefish. Ph.D. thesis, University of Massachusetts, Amherst, Mass.

Friedland, K.D., G.C. Garmon, A.J. Bejda, A.L. Studholme, and B. Olla. 1988. Interannual variation in diet and condition in juvenile bluefish during estuarine residency. Trans. Am. Fish. Soc. 117: 474–479.

Gardinier, M.N., and J.B. Hoff. 1982. Diets of striped bass in the Hudson River estuary. N.Y. Fish Game J. 29: 152–165.

Hartman, K.J. 1993. Striped bass, bluefish, and weakfish in the Chesapeake Bay: energetics, trophic linkages, and bioenergetics model applications. Ph.D. thesis, The University of Maryland, College Park, Md.

Hartman, K.J., and S.B. Brandt. 1993. Systematic sources of bias in a bioenergetics model: examples for age 0 striped bass. Trans. Am. Fish. Soc. 122: 912–926.

Hartman, K.J., and S.B. Brandt. 1995*a*. Trophic resource partitioning, diets, and growth of sympatric estuarine predators. Trans. Am. Fish. Soc. 124: 520–537.

Hartman, K.J., and S.B. Brandt. 1995*b*. Estimating energy density of fish. Trans. Am. Fish. Soc. 124: 347–355.

Hartman, K.J., and F.J. Margraf. 1992. Effects of prey and predator abundances on prey consumption and growth of walleyes in western Lake Erie. Trans. Am. Fish. Soc. 121: 245–260.

Hewett, S.W., and B.L. Johnson. 1992. A generalized bioenergetics model of fish growth for microcomputers, version 2.0. University of Wisconsin Sea Grant Institute, Madison, Wis.

Kendall, A.W., and L.W. Walford. 1979. Sources and distribution of bluefish, *Pomatomus saltatrix*, larvae and juveniles off the east coast of the United States. Fish. Bull. 77: 213–227.

Kitchell, J.F., and J.E. Breck. 1980. Bioenergetics model and foraging hypothesis for sea lamprey (*Petromyzon marinus*). Can. J. Fish. Aquat. Sci. 37: 2159–2168.

Kitchell, J.F., J.F. Koonce, R.V. O'Neill, H.H. Shugart, Jr., J.J. Magnuson, and R.S. Booth. 1974. Model of fish biomass dynamics. Trans. Am. Fish. Soc. 103: 786–798.

Kitchell, J.F., D.J. Stewart, and D. Weininger. 1977. Applications

of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 34: 1922–1935.

Klyashtorin, L.B., and A.A. Yarzhombek. 1975. Some aspects of the physiology of the striped bass, *Morone saxatilis*. J. Ichthyol. (Engl. Transl.), 15: 985–989.

Kohlenstein, L.C. 1981. On the proportion of the Chesapeake Bay stock of striped bass that migrates into the coastal fishery. Trans. Am. Fish. Soc. 110: 168–179.

Kruger, R.L., and R.W. Brocksen. 1978. Respiratory metabolism of striped bass, *Morone saxatilis* (Walbaum), in relation to temperature. J. Exp. Mar. Biol. Ecol. 31: 55–66.

Lucas, M.C., I.G. Priede, J.D. Armstrong, A.N.Z. Gindy, and L. DeVera. 1991. Direst measurements of metabolism, activity and feeding behavior of pike, *Esox lucius* L., in the wild, by use of heart rate telemetry. J. Fish Biol. 39: 325–345.

Lucas, M.C., A.D.F. Johnstone, I.G. Priedel. 1993. The use of physiological telemetry as a method of estimating metabolism of fish in the natural environment. Trans. Am. Fish. Soc. 122: 822–833.

Madon, S.P., and D.A. Culver. 1993. Bioenergetics model for larval and juvenile walleye: an in-situ approach using experimental ponds. Trans. Am. Fish. Soc. 122: 797–813.

Manooch, C.S., III. 1973. Food habits of yearling and adult striped bass, *Morone saxatilis* (Walbaum) from Albemarle Sound, North Carolina. Chesapeake Sci. 2: 142–205.

Mercer, L.P. 1983. A biological and fisheries profile of weakfish, *Cynoscion regalis*. Spec. Sci. Rep. No. 39. North Carolina Department of Natural Resources and Community Development, Division of Fisheries, Moorehead City, N.C.

Merriner, J.V. 1975. Food habits of the weakfish, *Cynoscion regalis*, in North Carolina waters. Chesapeake Sci. 16: 74–76.

Mid-Atlantic Fishery Management Council, Atlantic States Marine Fisheries Service, National Marine Fisheries Service, New England Fishery Management Council, and South Atlantic Fishery Management Council. 1989. Fishery management plan for the bluefish fishery. Mid-Atlantic Fishery Management Council, Dover, Del.

Minton, J.W., and R.B. McLean. 1982. Measurements of growth and consumption of sauger (*Stizostedion canadense*): implications for fish energetics studies. Can. J. Fish. Aquat. Sci. 39: 1396–1403.

Moore, C.M. 1988. Food habits, population dynamics, and bioenergetics of four predator fish species in Smith Mountain Lake, Virginia. Ph.D. thesis, Virginia Polytechnical Institute and State University, Blacksburg, Va.

Moore, C.M., J.J. Ney, and R.J. Neves. 1993. Parameter valuation of a bioenergetics model for striped bass. Publ. No. VSG-93-02,Virginia Sea Grant College Program, Charlottesville, Va.

Ney, J.J. 1990. Trophic economics in fisheries: assessment of demand–supply relationships between predators and prey. Aquat. Sci. 2: 55–81.

Norcross, J.J., S.L. Richardson, W.H. Massmann, and E.B. Joseph. 1974. Development of young bluefish (*Pomatomus saltatrix*) and distribution of eggs and young in Virginia coastal waters. Trans. Am. Fish. Soc. 103: 477–497.

Olla, B.L., and A.L. Studholme. 1971. The effects of temperature on the activity of bluefish, *Pomatomus saltatrix* L. Biol. Bull. (Woods Hole), 141: 337–349.

Olla, B.L., and A.L. Studholme. 1972. Daily and seasonal rhythms of activity in the bluefish (*Pomatomus saltatrix*). *In* The behavior of marine animals, Vol. 2. Vertebrates. *Edited by* H.E. Winn and B.L. Olla. Plenum Press, New York. pp. 303–326.

Raney, E.C. 1952. The life history of the striped bass, *Roccus saxatilis* (Walbaum) in tributaries of Chesapeake Bay. *In* Contributions to the study of subpopulations of fishes. U.S. Fish. Wildl. Serv. Spec. Sci. Rep. Fish. No. 208. pp. 85–107.

Rice, J.A., and P.A. Cochran. 1984. Independent evaluation of a bioenergetics model for largemouth bass. Ecology, 65: 732–739.

Rice, J.A., J.A. Breck, S.M. Bartell, and J.F. Kitchell. 1983. Evaluating the constraints of temperature, activity, and consumption on growth of largemouth bass. Environ. Biol. Fish. 9: 263–275.

Rudstam, L.G. 1988. Exploring the dynamics of herring consumption in the Baltic: applications of an energetics model of fish growth. Kiel. Meeresforsch. Sonderh. 6: 312–322.

Setzler, E.M, W.R. Boynton, K.V. Wood, H.H. Zion, L. Lubbers, N.K. Mountford, P. Frere, L. Tucker, and J.A. Mihursky. 1980. Synopsis of biological data on striped bass, *Morone saxatilis* (Walbaum). NOAA Tech. Rep. NMFS Circ. No. 433. FAO Synopsis No. 121.

Shuter, B.J., and J.R. Post. 1990. Climate, population viability, and the zoogeography of temperate fishes. Trans. Am. Fish. Soc. 119: 314–336.

Stewart, D.J., and F.P. Binkowski. 1986. Dynamics of consumption and food conversion by Lake Michigan alewives: an energetics-modeling synthesis. Trans. Am. Fish. Soc. 115: 643–661.

Stewart, D.J., and M. Ibarra. 1991. Predation and production by salmonine fishes in Lake Michigan, 1978–88. Can. J. Fish. Aquat. Sci. 48: 909–922.

Stewart, D.J., J.F. Kitchell, and L.B. Crowder. 1981. Forage fish and their salmonid predators in Lake Michigan. Trans. Am. Fish. Soc. 110: 751–763.

Stewart, D.J., D. Weininger, D.V. Rottiers, and T.A. Edsall. 1983. An energetics model for lake trout, *Salvelinus namaycush*: application to the Lake Michigan population. Can. J. Fish. Aquat. Sci. 40: 681–698.

Swenson, W.A., and L.L. Smith, Jr. 1973. Gastric digestion, food consumption, feeding periodicity, and conversion efficiency in walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 32: 33–41.

Thornton, K.W., and A.S. Lessem. 1978. A temperature algorithm for modifying biological rates. Trans. Am. Fish. Soc. 107: 284–287.

Tuncer, H., R.M. Harrell, and E.D. Houde. 1990. Comparative energetics of striped bass (*Morone saxatilis*) and hybrid (*M. saxatilis* × *M. chrysops*) juveniles. Aquaculture, 86: 387–400.

Wahl, D.H., and R.A Stein. 1991. Food consumption and growth of three esocids: field tests of a bioenergetics model. Trans. Am. Fish. Soc. 120: 230–246.

Wilk, S.J. 1979. Biological and fisheries data on weakfish, *Cynoscion regalis* (Bloch and Schneider). Tech. Ser. Rep. No. 21, Sandy Hook Laboratories, Highlands, N.J.

Winberg, G.G. 1956. Rate of metabolism and food requirements of fishes. Belorussian University, Minsk. (Transl. Fish. Res. Board Can., Transl. Ser. No. 194, 1960).

Zastrow, C.E., and E.D. Houde. 1990. Growth, oxygen uptake, energetics, and nitrogen utilization by young-of-the-year striped bass and striped bass hybrids. Final report (UMCEES-CBL-90-014) to Maryland Sea Grant, College Park, Md.

Appendix

We show how to implement the bioenergetics models with exponential temperature-dependent respiration functions and constant ACT in the Hewett and Johnson (1992) model.

To use the bioenergetics models for striped bass, bluefish, and weakfish in the Hewett and Johnson (1992) software

package requires modification of input variables in Tables 1–3 because these models use an exponential temperature function and constant activity multiplier in the respiration models. In Hewett and Johnson (1992) the intercept RA, weight coefficient RB, and temperature coefficient RQ are the same as presented in Tables 1–3. To make a constant ACT in respiration model 1, Hewett and Johnson's (1992) variables RTM, RTL, RK4, and BACT should be set to 0. Hewett and Johnson's (1992) variables RK1 and ACT are set to 1 and RTO is the natural logarithm of ACT in Tables 1–3 in this paper. For example, in the bluefish model the mean ACT in Table 2 is 1.8805. In the Hewett and Johnson (1992) model, RTO would be the natural log of 1.8805 or 0.6315.

Predatory demand and impact of striped bass, bluefish, and weakfish in the Chesapeake Bay: applications of bioenergetics models

Kyle J. Hartman and Stephen B. Brandt

Abstract: We applied bioenergetics models for dominant Chesapeake Bay piscivores, striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*), and weakfish (*Cynoscion regalis*), along with site-specific data on diets, growth, and energy density, to examine trophic linkages and the relationship of predatory demand to prey supply. Atlantic menhaden (*Brevoortia tyrannus*), bay anchovy (*Anchoa mitchilli*), and spot (*Leiostomus xanthurus*) accounted for 65–99% of the annual biomass of piscivore diets (excluding age-0 striped bass that ate mostly invertebrates). The diets of young piscivores were dominated by anchovy, but menhaden and spot became increasingly important to older fish. Young (age < 2) striped bass ate mostly benthic prey. Older striped bass fed increasingly on pelagic sources, primarily menhaden, but bluefish and weakfish increased benthic resource use from 10% at age 0 to 50% by age 2. Comparison of consumption (supply) to demand (potential consumption) measured the suitability of Chesapeake Bay for predator production. Bluefish came closest to achieving their demand for prey, suggesting that they are more successful predators than either striped bass or weakfish. Results suggest that Chesapeake Bay may be a better nursery than production area for older fish, and prey supply (not temperature) may account for the movements and use of the estuary by older piscivores.

Résumé : Nous avons appliqué des modèles bioénergétiques aux piscivores dominants de la baie Chesapeake, soit le bar d'Amérique (*Morone saxatilis*), le tassergal (*Pomatomus saltatrix*) et l'acoupa royal (*Cynoscion regalis*), et nous avons pris des données caractéristiques d'endroits déterminés et qui portent sur le régime alimentaire, la croissance et la densité énergétique, afin d'examiner les liens trophiques et le rapport entre la demande et l'offre de proies. L'alose tyran (*Brevoortia tyrannus*), l'anchois américain (*Anchoa mitchilli*) et le tambour croca (*Leiostomus xanthurus*) correspondent à une fraction comprise entre 65 et 99% de la biomasse consommée annuellement par ces piscivores (à l'exclusion du bar d'Amérique de moins d'un an, qui se nourrit surtout d'invertébrés). Le régime alimentaire des jeunes piscivores est constitué surtout de l'anchois, mais l'alose et le tambour comptent de plus en plus dans le régime à mesure que le poisson grossit. Les jeunes bars (de moins de 2 ans) se nourrissent principalement de proies benthiques. Chez les bars plus âgés, les ressources pélagiques, surtout l'alose, sont de plus en plus exploitées avec l'âge alors que le tassergal et l'acoupa font une utilisation croissante des ressources benthiques, de 10% chez les sujets de moins d'un an à 50% chez ceux de 2 ans. La comparaison de la consommation (offre) à la demande (consommation potentielle) permet de déterminer dans quelle mesure la baie Chesapeake convient à la production de prédateurs. Des trois, le tassergal est l'espèce qui parvient le mieux à combler sa demande en proies; cela donne à penser que ce sont des prédateurs mieux adaptés à leur milieu que les deux autres espèces. Les résultats indiquent que la baie Chesapeake serait meilleure à titre d'aire d'élevage que d'aire de production pour les poissons plus âgés, et que l'offre de proies (plutôt que la température) pourrait constituer l'explication des déplacements par les piscivores plus âgés et de leur utilisation de l'estuaire.

[Traduit par la Rédaction]

Received November 17, 1994. Accepted March 3, 1995.
J12165

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Introduction

The striped bass, *Morone saxatilis*, has historically been a dominant predator as well as an important commercial and recreational fish in the Chesapeake Bay. Commercial landings of striped bass peaked in 1973 at over 6300 metric tons (t) but by 1983 landings had declined to 772 t (Mid-Atlantic Fishery Management Council 1984). In 1985, stringent management measures were enacted to curtail the decline in striped bass numbers and striped bass stocks have subsequently increased dramatically. The present striped bass population now permits a limited sport and commercial season in the Chesapeake Bay.

As the striped bass population recovered, concern was raised over whether striped bass may be placing predatory pressure on other valued species. As the population grows, production of striped bass will ultimately be limited by the available production of prey species. Striped bass have been suggested as being competitors with bluefish, *Pomatomus saltatrix*, and weakfish, *Cynoscion regalis* (Setzler et al. 1980; Mercer 1983; Mid-Atlantic Fishery Management Council et al. 1989); however, there have been no studies conducted to determine the forage requirements of these species in estuarine waters.

Clearly there is a need to understand the predatory demand of striped bass, bluefish, and weakfish in the Chesapeake Bay. These piscivores are the top predators in the system and as such, have the potential to regulate energy flow in lower trophic levels (top-down control) or to be influenced by changes in production at lower trophic levels (bottom-up control) (Carpenter et al. 1985, 1987).

Bioenergetics models are being used widely in fisheries management and ecology to compare prey production with predator consumption (Stewart et al. 1981, 1983; Stewart and Ibarra 1991; Hartman and Margraf 1992; LaBar 1993), to predict periods of peak host mortality caused by sea lamprey, *Petromyzon marinus* (Kitchell and Breck 1980), to evaluate predator–prey interactions (Lyons and Magnuson 1987; Hartman and Margraf 1993), to describe the accumulation of toxicants in fish (Norstrom et al. 1976; Connolly and Tonelli 1985; Barber et al. 1991; Borgmann and Whittle 1992), and to predict predator growth potential on spatial scales (Brandt et al. 1991, 1992; Goyke and Brandt 1993). Bioenergetics models are robust estimators of consumption from growth information (Kitchell et al. 1977; Bartell et al. 1986; Hewett 1989) and have been validated for most of the species for which they have been attempted (Minton and McLean 1982; Cochran and Rice 1982; Rice and Cochran 1984; Beauchamp et al. 1989; Hartman and Brandt 1993, although see Ney 1990 and Wahl and Stein 1991).

The purpose of this paper is to use the bioenergetics models developed and evaluated in Hartman and Brandt (1995*c*) for striped bass, bluefish, and weakfish, to address a fundamental management and ecological question: What are the predatory demands of striped bass, bluefish, and weakfish in the Chesapeake Bay? Further modeling analysis evaluates predator demand and prey supply in Chesapeake Bay.

Methods

Bioenergetics models for striped bass, bluefish, and weakfish were developed in Hartman and Brandt (1995*c*). These models were run in the MS-DOS system with the fish bioenergetics model (v. 2.0) for microcomputers distributed by Wisconsin Sea Grant (Hewett and Johnson 1992). In addition to the species-specific physiological variables, the Hewett and Johnson (1992) model requires specific inputs for the temperature occupied by the fish, the energy density of predators and prey, and the diet proportions for each prey type throughout the modeling period. Modeling periods in this software package can be set from 1 to 365 days with a daily time step. We used temperature data collected from a depth of 0.5 m at the research pier of Chesapeake Biological Laboratory, Solomons, Maryland, between July 1989 and March 1992, and averaged for 7-d periods across all years (Fig. 1). We considered this temperature data to be representative of the thermal experiences of the predators in Chesapeake Bay because little vertical thermal structure is found in the oxygenated zones of mid-Chesapeake Bay where this study took place and because research pier temperatures followed those of mid-bay stations closely during 1991 and 1992 (K. Hartman, unpublished data). Energy density of predators and their prey was determined seasonally by drying fish collected in the field (during 1990–1992) from each piscivore cohort or prey species. After the fish were dried to a constant weight in a 70°C oven, the resulting dry weight as a percentage of wet weight was applied to species-specific or generic models of fish energy density (Hartman 1993) to determine the mean energy density for piscivores (Tables 1 and 2) and prey fish (Table 3).

Diet and growth data from Hartman (1993) and Hartman and Brandt (1995*a*) from fish collected in Chesapeake Bay during 1990–1992 were used to estimate consumption with the bioenergetics models. Diets were summarized by measuring the top 6–10 prey types for these analyses (Tables 4–6). Growth of predators was generalized within an age-class to minimize individual variability by using mean wet weights throughout the year for striped bass or with logistic equations best fit by least squares to the cohort-specific

Fig. 1. Average daily water temperatures used for bioenergetics simulations. Temperatures were recorded from a depth of 0.5 m at the research pier at Chesapeake Biological Laboratory, Solomons, Maryland, near the mouth of the Patuxent River, Maryland, between 1 July 1989 and 31 March 1993 (H. Millsaps, unpublished data).

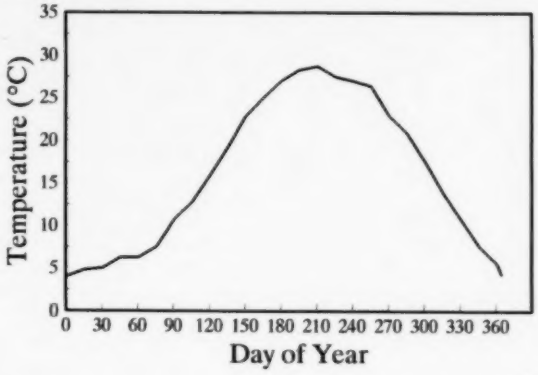


Table 1. Seasonal energy density (cal·g wet weight^{-1}; 1 cal = 4.184 J) of weakfish and bluefish for use in the bioenergetics models.

	Weakfish						
	Age 0					Bluefish	
Day	*a*	*b*	*c*	Age 1	Age 2	Age 0	Age 1
150	793	647	485	913	1500	646	1359
165	830	678	508	983	1500	719	1392
180	869	710	532	1057	1385	801	1426
195	911	743	557	1137	1225	892	1460
210	954	778	584	1223	1160	993	1495
225	999	815	611	1316	1100	1105	1531
240	1046	854	640	1416	1175	1230	1568
255	1096	894	671	1523	1200	1369	1606
270	1147	937	703	1639	1400	1524	1645
285	1202	981	736	1763	1500	1697	1685
300	1259	1027	771	1896	1600	1889	1725
315	1318	1076	807	2040	1700	2103	1767

Note: Energy densities are calculated from species-specific and generic models of fish energy density (Hartman 1993) and seasonal means of percent dry weight for each species and age-class with interpolation between observations. Age-0 weakfish cohorts *a*, *b*, and *c* correspond to fish that achieved 1 g wet weight by July 1, August 1, and September 1, respectively.

Table 2. Seasonal energy density (cal·g wet weight^{-1}; 1 cal = 4.184 J) of striped bass for use in the bioenergetics models.

	Striped bass			
Day	Age 0	Age 1	Age 2	Age 3+
1		1382	1411	1610
15		1383	1468	1755
30		1384	1525	1900
45		1386	1613	1905
60		1387	1700	1910
75		1388	1775	1915
90		1389	1850	1920
105		1391	1825	1900
120		1392	1800	1880
135		1393	1787	1880
150	932	1394	1775	1880
165	963	1395	1767	1880
180	995	1397	1760	1880
195	1028	1398	1695	1865
210	1062	1399	1630	1850
225	1097	1400	1620	1860
240	1133	1401	1610	1870
255	1171	1403	1525	1835
270	1210	1404	1440	1800
285	1250	1405	1385	1785
300	1292	1406	1330	1770
315	1335	1407	1353	1765
330	1379	1409	1377	1760
345	1425	1410	1400	1765
360	1472	1411	1505	1770
365	1488	1411	1610	1775

Note: Energy densities are calculated from species-specific and generic models of fish energy density (Hartman 1993) and seasonal means of percent dry weight for each age-class with interpolation between observations.

individual weight data of weakfish and bluefish. Fish weights were manually entered into the Hewett and Johnson (1992) bioenergetics model from spreadsheet estimates of weight versus day. The modeling year was based upon the calendar year with model day 1 equal to January 1 and day 365 equal to December 31. Additional verification of weights attained by the end of the year (final weights) for age-classes of striped bass was obtained by applying a fall–winter length–weight relationship to November–December mean total lengths from Maryland Department of Natural Resources bay-wide stock assessments (Maryland Department of Natural Resources 1989). We averaged the weights of predators within a cohort for 4–9 time periods within a year and interpolated weights between point estimates. The resulting seasonal growth patterns of predators are shown in Figs. 2–4 (Hartman and Brandt 1995*a*).

Predatory consumption and predatory demand

Predatory demand (amount of food consumed under ad libitum conditions, given the ambient temperatures, energy densities, and fish wet weight) of several cohorts of striped bass, bluefish, and weakfish during estuarine residency was modeled. The period of estuarine residency was considered to be 365 d for age-1 through age-6 striped bass, 183 d for age-0 striped bass (day 183 through day 365), 126 d for age-0 bluefish and weakfish, and 155 d for age-1 and age-2 bluefish and weakfish (day 154 through day 308). These specific dates for estuarine residency were chosen because they represent the mean dates when each species and cohort was first captured and typically last captured during field collections (Hartman 1993). Mean dates at first capture differed by only several days across species for age-0 fish, and across bluefish and weakfish for age ≥ 1 fish, so dates were averaged to day 183 and day 154 for age 0 and age ≥ 1, respectively, to make across-species comparisons simpler.

All striped bass and bluefish age-classes were modeled as individual cohorts; however, given the batch spawning and protracted recruitment of weakfish (Szedlmayer et al. 1990), we modeled weakfish with two to three cohorts per age-class. Determination of the exact numbers of weakfish cohorts present within an age-class would not have been possible without examining daily increments in age-0 fish. Cohort determination in age-1 and older weakfish is not possible even with otolith techniques. Examination of length frequencies for weakfish did not suggest any simple method of differentiating weakfish cohorts. Therefore, for age-0 weakfish, three cohorts (*a*, *b*, and *c*) were established. Each age-0 cohort began the modeling period at 1 g with model periods of day 183 through day 308 for cohort *a*, day 210 through day 308 for cohort *b*, and

Table 3. Seasonal energy density (cal·g wet weight^{-1}; 1 cal = 4.184 J) of prey for use in the bioenergetics models.

	Day							
	1	60	121	182	244	305	365	*N*
Bay anchovy[a]	1096	1060	1025	991	957	925	895	147
Atlantic menhaden[b]	1100	1100	1100	940	1003	1061	1098	91
Atlantic silverside[b]	1016	1060	1101	1145	1189	1232	1274	89
Spot[b]	2520	2520	1974	1712	1545	1427	1341	38
Atlantic croaker[b]	1726	1726	771	1233	1726	1726	1726	73
White perch[b]	1431	1431	1593	1695	1773	1834	1885	28
Naked goby[b]	716	716	716	716	716	716	716	146
Other fish[b]	1202	1315	1251	1200	1197	1199	1202	—
Polychaetes[c]	849	849	849	849	849	849	849	—
Blue crab[c]	923	923	923	923	923	923	923	—
Neomysis spp.[c]	1151	1151	1151	1151	1151	1151	1151	—
Gammarids[c]	986	986	986	986	986	986	986	—
Clams[c]	548	548	548	548	548	548	548	—
Copepods[c]	550	550	550	550	550	550	550	—
Other invertebrates[c]	750	750	750	750	750	750	750	—

Note: Energy densities of fish are calculated from species-specific and generic models of fish energy density (Hartman and Brandt 1995*b*) and seasonal means of percent dry weight (Hartman 1993) with interpolation between observations for each species. Energy densities of all invertebrates were averages reported in Cummins and Wuycheck (1971).

[a]Species-specific energy density model.

[b]Generic energy density model.

[c]Reported in Cummins and Wuycheck (1971).

day 238 through day 308 for cohort *c*. Final weights for each cohort were set near the upper (cohort *a*), middle (cohort *b*), and lower (cohort *c*) ranges of end of year weights observed for age-0 weakfish (Fig. 4; data from Hartman and Brandt 1995*a*).

Growth data for age-1 and older weakfish were highly variable, but appeared to indicate at least two size groups within an age-class for a given time of year. Therefore, we continued to use multiple cohorts within an age-class for modeling weakfish consumption, but restricted the analysis to a larger (cohort *a*) and smaller cohort (cohort *b*) for age-1 and age-2 weakfish.

In estimating consumption and predatory demand, a fine time scale (14 d for periods between June and November) was used for growth inputs to enable more accurate model predictions of consumption dynamics. Short time periods in bioenergetics models have been shown to provide the most realistic and accurate reflections of prey use by fish (Rice and Cochran 1984; Cochran and Knutsen 1988; Hewett and Johnson 1992). The Hewett and Johnson (1992) model permits a maximum of 20 growing periods in annual runs of the bioenergetics model. We sought to take advantage of the maximum number of growth periods (20), but for simplicity and consistency we also wanted to keep the number of days used in modeling periods uniform across species and cohorts. We used 20 growing periods (the year was divided into 20 growth stanzas) for age-1 and older striped bass; 13 growing periods for age-0 striped bass; 11 growing periods for age-1 and older weakfish and bluefish; 9, 5, and 7 growing periods for cohorts *a*, *b*, and *c* of age-0 weakfish, and 9 growing periods for age-0 bluefish modeling.

Individual consumption was modeled for both the observed growth rates and the potential growth rates if fish had fed at maximum levels (predator demand) under the prevailing thermal conditions. The comparison of prey consumed based on observed growth in the field was equivalent to a measure of prey supply for each species. The consumption estimates from fish feeding at maximum levels provided a measure of predator demand for prey (Ney 1990). Comparison of these two levels of consumption through time allows detection of possible periods when prey availability declines and helps to establish what factors (e.g., temperature, prey availability) may limit piscivore production in the Chesapeake Bay.

We modeled consumption by individuals of each age-class and for hypothetical populations of each predator species. No absolute estimates of population-level consumption were possible because no population estimates are available for striped bass, bluefish, or weakfish in the Chesapeake Bay. However, we believed that modeling of hypothetical predator populations might provide valuable insights into the relative roles of the predators in affecting prey fish populations owing to ontogenetic differences in diets, consumption, growth, and fishing mortalities. Therefore, we modeled consumption by each hypothetical population by assuming an initial number of 10 million fish each weighing 1 g (the beginning weight for age-0 modeling) for each cohort of each species. The number of fish in age-1 and older cohorts was a product of survival

Table 4. Seasonal diet summaries (percent biomass) used to partition consumption among prey types for striped bass in the bioenergetics models.

(A) Age 0

Day	Naked goby	Other fish	Polychaetes	Blue crab	*Neomysis* spp.	Gammarids	Clams	Other invertebrates
151	0	0	15.7	0	0.5	83.8	0	0
212	39.0	19.9	8.6	1.3	13.3	3.6	0	14.3
273	0	0	76.1	0	14.1	9.7	0	0.1
334	4.4	0.1	0.5	0.1	27.3	0.5	65.7	1.4
365	4.4	0.1	0.5	0.1	27.3	0.5	65.7	1.4

(B) Age 1

Day	Bay anchovy	Atlantic menhaden	Spot	Naked goby	Other fish	Copepods	*Neomysis* spp.	Gammarids	Clams	Other invertebrates
1	0.3	0	0	0	0	69.4	2.5	11.5	14.9	1.3
90	16.0	0	0	0.8	2.3	0.6	52.4	14.3	4.2	9.4
151	10.1	0	14.4	8.8	13.1	0	13.6	29.0	0	10.9
212	31.0	22.5	0	0	1.2	0	27.3	0.8	0.2	17.0
273	23.1	52.7	0	2.6	4.9	0	1.0	0	0	15.8
334	24.2	56.8	0	2.9	6.0	0	0.1	0.5	1.1	8.4
365	24.2	56.8	0	2.9	6.0	0	0.1	0.5	1.1	8.4

(C) Age 2

Day	Bay anchovy	Atlantic menhaden	Spot	Atlantic silverside	Other fish	Polychaetes	Blue crab	Other invertebrates
1	11.0	79.2	0	3.4	4.6	0.1	0	1.7
31	11.0	79.2	0	3.4	4.6	0.1	0	1.7
90	22.2	59.5	0	0.4	0.7	5.1	0	12.0
151	0	0	84.8	0	11.9	0.6	2.3	0.4
212	27.2	23.9	0	18.5	0.1	0.4	27.6	2.4
273	4.6	35.4	0	42.1	11.4	0.8	2.1	3.7
335	0	98.1	0	0	0	0	0	1.9
365	0	98.1	0	0	0	0	0	1.9

(D) Age ≥ 3

Day	Bay anchovy	Atlantic menhaden	Spot	Atlantic silverside	White perch	Other fish	Polychaetes	Other invertebrates
1	5.0	64.2	0	0.5	25.0	5.2	0	0.1
31	5.0	64.2	0	0.5	25.0	5.2	0	0.1
91	3.5	86.1	0	0	0.1	1.8	6.3	2.2
151	1.9	8.4	42.5	2.5	0.1	11.0	31.5	2.2
212	20.9	51.3	0	1.0	0	2.6	22.6	1.5
273	0.2	96.6	0	2.3	0	0	0.1	0.8
334	0	99.1	0	0	0	0.6	0	0.3
365	0	99.1	0	0	0	0.6	0	0.3

Note: Data are from Hartman and Brandt (1995*a*).

from age 0 to age 1 and from age 1 to age 2 for bluefish and weakfish (Ricker 1975). For striped bass, the population was structured by continuing the survival schedule from one age-class to the next through age 6 (the oldest cohort modeled).

To structure the hypothetical predator populations required information on the natural and fishing mortalities of each species. Although a general age-dependent natural mortality regression already exists for North Sea fishes, it was not used because it does not apply for fish less

Table 5. Seasonal diet summaries (percent biomass) used to partition consumption among prey types for bluefish in the bioenergetics models.

(A) Age 0

Day	Bay anchovy	Atlantic menhaden	Atlantic silverside	Spot	Other fish	Other invertebrates
151	99.7	0	0	0	0	0.3
212	81.5	0	14.6	0	3.9	0
273	58.7	30.6	3.3	6.8	0.1	0.5
365	58.7	30.6	3.3	6.8	0.1	0.5

(B) Age 1

Day	Bay anchovy	Atlantic menhaden	Atlantic silverside	Spot	Other fish	Other invertebrates
1	30.9	9.9	8.5	35.8	14.3	0.7
212	30.9	9.9	8.5	35.8	14.3	0.7
273	12.0	66.1	0.1	18.9	2.8	0.1
365	12.0	66.1	0.1	18.9	2.8	0.1

(C) Age 2

Day	Bay anchovy	Atlantic menhaden	Atlantic croaker	Spot
1	0.6	88.0	0	11.4
212	26.4	3.7	62.8	7.1
273	0.6	88.0	0	11.4
365	0.6	88.0	0	11.4

Note: Data are from Hartman and Brandt (1995*a*).

than 1 year old (Boudreau and Dickie 1989). A similar length-based mortality model for striped bass was not used for structuring the hypothetical populations because it dealt only with mortality rates from larvae through the first year of life (Logan 1985). Similar size-specific mortality data were lacking for weakfish and bluefish; therefore, we applied a weight-specific mortality equation for striped bass to populations of all three predator species. Natural mortality rates of striped bass were obtained from the literature (Chadwick 1968; Dey 1981; Dorazio et al. 1991) and modeled as a function of weight. The resulting regression equation ($N = 7$; $r^2 = 0.94$) was

$$M = 0.036\ 17\ W^{-0.537\ 82}$$

where M is the instantaneous natural mortality rate and W is wet weight (g). This size-based natural mortality relationship was used in a spreadsheet with the seasonal weights of each predator species and cohort (Figs. 2–4; or see Tables 16–18 in Hartman 1993) to calculate the mortality rate during a modeling interval. For example, an average individual of the age-0 cohort of striped bass grew from 1 g on day 183 to 2.3 g on day 197. The mean weight during this interval is 1.65 g. The mean weight is applied to the mortality regression equation above and M is estimated as 0.027 63. In bioenergetics modeling applications with the Hewett and Johnson (1992) model we applied such a mortality estimate for the entire interval (day 183 to day 197). The initial population numbers for the first growing interval were 10 million fish. The initial population numbers for the second growth interval were derived in the spreadsheet using the average M for the interval (0.027 63) applied to a population size of 10 million for 14 d. Mortality estimates and initial population sizes for subsequent growing intervals (and age-classes) were determined in an analogous manner.

Fishing mortality rates were obtained from the literature and management agencies to structure the populations of the hypothetical predator populations. Striped bass fishing mortality ($F = 0.23$/year, Goshorn 1993) was restricted to the fall–winter period from day 295 through day 365 ($F = 0.003\ 68$/d) and was applied only to cohorts that had exceeded the 18-inch (1 inch = 25.4 mm) minimum size limit (age 4 through age 6). Fishing mortality rates for bluefish were initiated on day 266 for age-0 fish when wet weights averaged 240 g ($F = 0.001\ 18$, Mid-Atlantic Fishery Management Council et al. 1989) and continued through age 2. Weakfish were considered partially recruited to the fishery at age 1 when mean weights of individuals within a cohort exceeded 100 g wet weight, and fully recruited at age 2 (Atlantic States Marine Fisheries Commission 1992). For age-1 weakfish over 100 g we assumed $F = 0.001\ 37$. For age-2 weakfish, $F = 0.002\ 74$ (Atlantic States Marine Fisheries Commission 1992).

As for bluefish and striped bass, the hypothetical weakfish population was modeled with a starting number of

Table 6. Seasonal diet summaries (percent biomass) used to partition consumption among prey types for weakfish in the bioenergetics models.

Age 0

Day	Bay anchovy	Atlantic menhaden	Atlantic croaker	Spot	Other fish	*Crangon* spp.	*Neomysis* spp.	Other invertebrates
151	73.7	0	0	0	0	11.1	13.0	2.3
212	73.7	0	0	0	0	11.1	13.0	2.3
273	97.9	0	0	0	0.2	0.1	1.5	0.2
334	58.2	0	37.4	0	0	0	4.4	0

Age 1

Day	Bay anchovy	Atlantic menhaden	Atlantic croaker	Spot	Other fish	*Crangon* spp.	*Neomysis* spp.	Other invertebrates
1	72.7	3.7	0	2.9	3.6	1.7	13.4	2.0
151	72.7	3.7	0	2.9	3.6	1.7	13.4	2.0
211	70.3	7.2	0.2	0	9.6	6.0	3.7	3.2
273	17.4	53.6	11.5	13.7	2.3	0.4	0.1	0.7
365	17.4	53.6	11.5	13.7	2.3	0.4	0.1	0.7

Age 2

Day	Bay anchovy	Atlantic menhaden	Atlantic croaker	Spot	Other fish	*Crangon* spp.	*Neomysis* spp.	Other invertebrates
1	7.8	22.1	1.7	43.2	14.2	2.1	0	8.9
151	7.8	22.1	1.7	43.2	14.2	2.1	0	8.9
273	0.9	64.2	0	30.4	4.4	0.1	0	0
365	0.9	64.2	0	30.4	4.4	0.1	0	0

Note: Data are from Hartman and Brandt (1995*a*).

10 million 1-g fish. However, weakfish spawn nearly continuously from May through August with peak spawning activity in the Chesapeake Bay occurring during early summer (June–July, Szedlmayer et al. 1990). Therefore, we modeled three different age-0 cohorts and began each cohort with 10 million fish. Each age-0 cohort corresponded to fish attaining 1 g wet weight on or about day 183 (cohort *a*), day 210 (cohort *b*), and day 239 (cohort *c*). We were able to discern only two cohorts from the growth data for age-1 and age-2 weakfish so we used only cohorts *a* and *b* for modeling consumption by older weakfish cohorts.

To examine trophic resource use by the predators, we pooled consumption estimates on the basis of their perceived trophic pathway (i.e., whether the prey fed mainly on benthic or pelagic prey). Pelagic pathways were those considered to be represented by species that feed predominantly in pelagic food webs such as bay anchovy (*Anchoa mitchilli*), Atlantic menhaden (*Brevoortia tyrannus*), and Atlantic silversides (*Menidia menidia*) (Homer and Boynton 1978; Klebasko 1991; Jeffries 1975). Benthic pathway prey sources were prey such as spot (*Leiostomus xanthurus*), Atlantic croaker (*Micropogonius undulatus*), naked goby (*Gobiosoma bosci*), blue crab (*Callinectes sapidus*), polychaetes, and gammarids that feed predominantly on benthos (Hixon and Heck 1978; Darnell 1958; Homer and Boynton 1978).

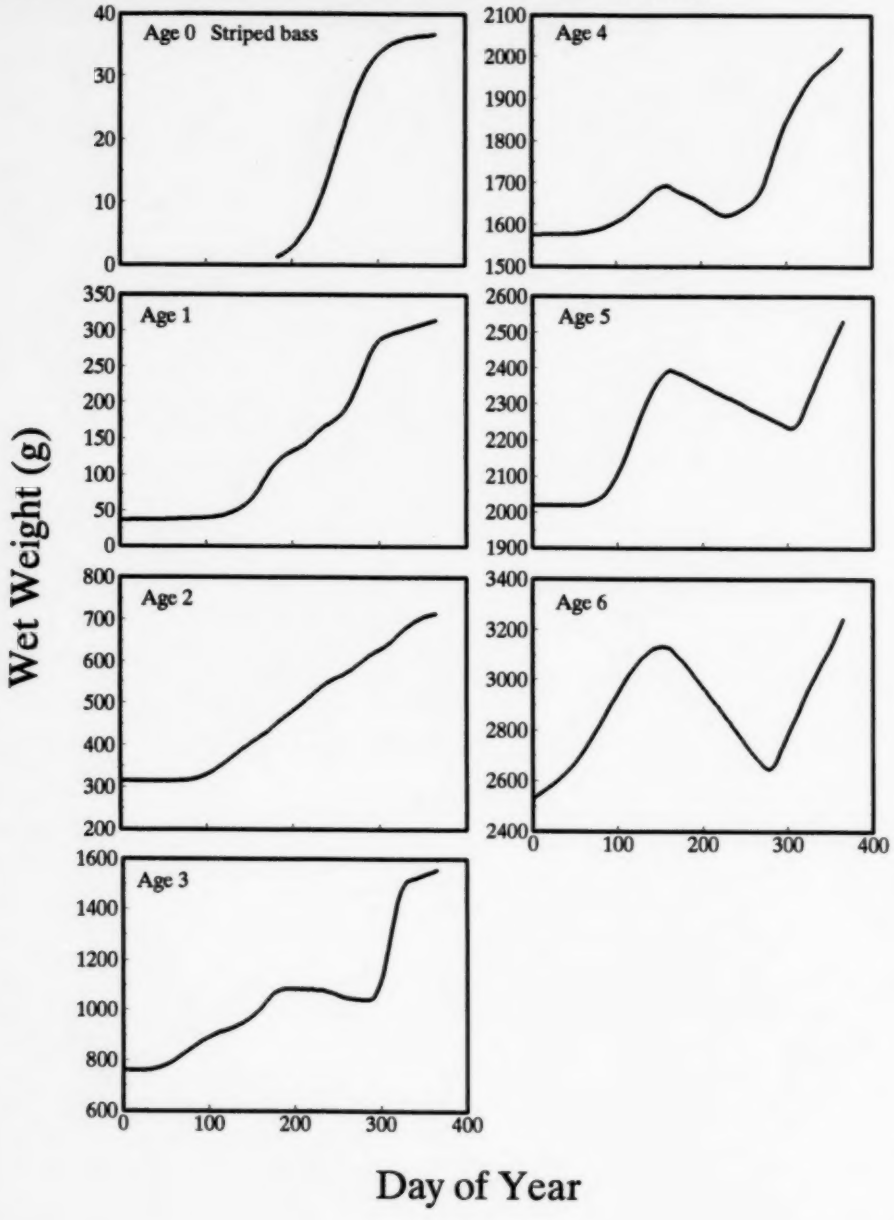
Results

Individual consumption

At equal ages, bluefish consumed more prey per individual than did striped bass or weakfish (Table 7, Figs. 5, 6). An age-0 bluefish would require over 2100 g of prey to grow as observed in the field. Cumulative consumption by an age-0 bluefish during the 126-d modeling period was approximately equal to that of an age-1 striped bass throughout the entire year (Table 7). Consumption by age-1 and age-2 bluefish was 5351 and 11 360 g, respectively. Consumption by age-1 bluefish was about the same as that by age-2 striped bass (4511 g) and age-2, cohort *a* weakfish (5652 g). Cumulative consumption by an individual age-2 bluefish was exceeded only by that of age-5 and age-6 striped bass (Table 7).

Consumption by individuals from striped bass age-classes was similar to that of like-aged weakfish from cohort *a* (Table 7). An individual age-0 striped bass consumed 192 g of prey, while an age-0 weakfish ate 54 g (cohort *c*) to 296 g (cohort *a*) of prey annually. Age-1 striped bass ate 2122 g of prey, compared with 2761 g and 1332 g for age-1 weakfish of cohorts *a* and *b*. Consumption by age-2 striped bass was 4512 g, intermediate to the values for cohort *a* (5652 g) and cohort *b* (3071 g) age-2 weakfish (Table 7).

Fig. 2. Seasonal growth patterns for age-0 through age-6 striped bass used in bioenergetics model estimates of consumption. Growth data are from Hartman and Brandt (1995*a*).



Relative annual prey use by piscivores was described by dividing the consumption of each prey type by the total annual consumption. Annual diets (percent of total biomass consumed) of striped bass changed with age (Fig. 7). Age-0 striped bass ate mostly polychaetes (38%) and other invertebrates (45%, primarily gammarids and *Neomysis* spp.). Age-1 striped bass continued to use invertebrates (34%) but became piscivorous, eating mostly Atlantic menhaden (33%) and bay anchovy (23%). The contribution of Atlantic menhaden to the production of striped bass increased from age 1 through age 3. Menhaden accounted for 33% of consumption by age-1, 42% of consumption by age-2, and 72% of consumption by age-3 striped bass. Atlantic menhaden continued to be important to age-4 through age-6 striped bass, contributing 63–66% of their annual consumption (Fig. 7). Polychaetes (12–13%) and spot (7–8%)

Fig. 3. Seasonal growth patterns for age-0 through age-2 bluefish used in bioenergetics model estimates of consumption. Growth data are from Hartman and Brandt (1995*a*).

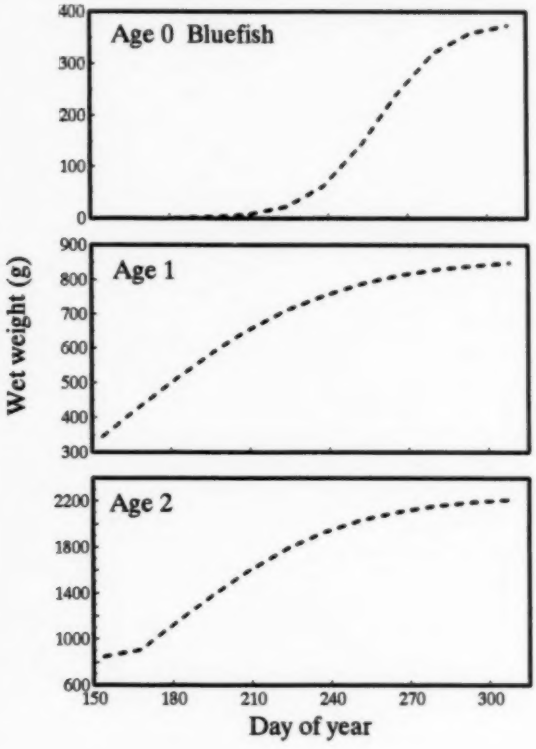
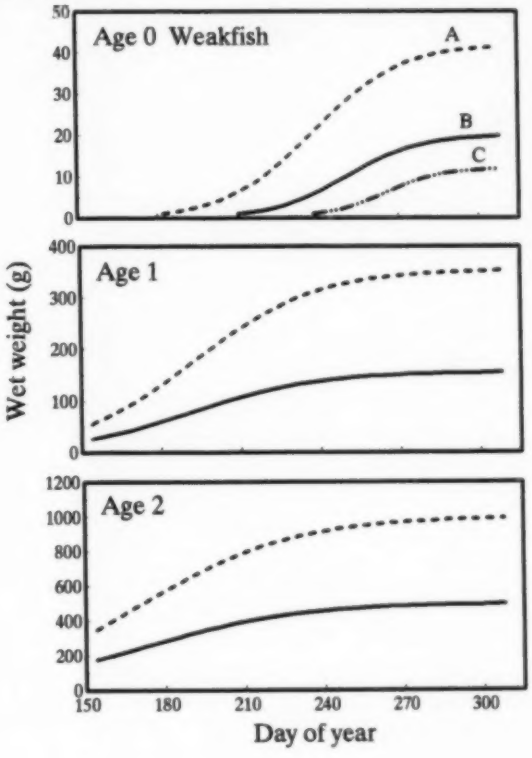


Fig. 4. Seasonal growth patterns for age-0 through age-2 weakfish used in bioenergetics model estimates of consumption. Cohort *a* (broken line, A) represents fish attaining 1 g wet weight by day 183 as age 0; cohort *b* (solid line, B) represents fish attaining 1 g wet weight by day 210 as age 0; and cohort *c* (chained line, C) represents fish attaining 1 g wet weight by day 239 as age 0. Growth data are from Hartman and Brandt (1995*a*).



also contributed to the production of age-4 through age-6 striped bass.

Bluefish annual diets were dominated by four prey species, bay anchovy, Atlantic menhaden, Atlantic croaker, and spot, but showed changes in the importance of the prey species with ontogeny (Fig. 8). The annual diet of age-0 bluefish was dominated by bay anchovy (63%) and to a lesser degree by Atlantic menhaden (24%). Age-1 bluefish increased their use of menhaden (33%) and decreased their use of anchovy (23%) relative to their age-0 conspecifics. Spot (29%) made up the majority of the remainder of age-1 diets. Atlantic menhaden (41%), Atlantic croaker (35%), bay anchovy (15%), and spot (9%) accounted for all consumption by age-2 bluefish (Fig. 8).

Despite different growth regimes, annual diets of cohorts within a weakfish age-class did not differ (Fig. 9). Annual diets of age-0 weakfish (all cohorts) were dominated by bay anchovy. Bay anchovy represented 87–90% of annual individual consumption for age-0 weakfish. *Neomysis* spp. were the next most common prey type in annual diets, representing 3–6% of annual consumption. Although diets among cohorts (within an age-class) were similar, the contribution by bay anchovy declined, while the contribution by menhaden and spot increased for older fish (Fig. 9). Age-1 weakfish annual diets were made up largely of bay anchovy (48%) and menhaden (27%). Spot (6%) were of minor importance to age-1 weakfish, but accounted for 35% of consumption for age-2 weakfish. Atlantic menhaden accounted for 50%, and bay anchovy only 3%, of annual consumption by age-2 weakfish (Fig. 9).

Owing to the high consumption rates of bluefish, the annual consumption (g/fish each year) of bay anchovy was higher for age-0 through age-2 bluefish than for all other piscivore cohorts except age-1 cohort *a* weakfish (Fig. 8). Individual bluefish ate 1242–1683 $g \cdot yr^{-1}$ of bay anchovy at age 0 through age 2. Although bay anchovy were not a high proportion of the annual diets of age-2 bluefish (15% of annual diet), by virtue of higher total consumption, an individual age-2 bluefish ate more anchovy than did individual weakfish of all cohorts and ages that relied more heavily on anchovy as prey (90% for age 0, 48% for age 1; Table 7).

Annual consumption of menhaden increased with age for all species (Figs. 5, 6). Age-3 and older striped bass ate more menhaden per year than did bluefish and weakfish

Table 7. Annual cumulative consumption ($g \cdot yr^{-1}$) for individual age-0 through age-2 bluefish and weakfish, and age-0 through age-6 striped bass estimated from bioenergetics models for each species.

Cohort	Bay anchovy	Atlantic menhaden	Atlantic silverside	Atlantic croaker	Spot	Other fish	Other invertebrates	Total
Striped bass								
Age 0	0	0	0	0	0	23	169	192
Age 1	451	714	0	0	24	133	629	1951
Age 2	525	1879	661	0	651	216	580	4512
Age 3	568	5859	108	0	634	463	1136	8768
Age 4	769	7294	145	0	773	665	1453	11099
Age 5	991	8629	171	0	1067	876	1915	13650
Age 6	1067	10610	187	0	1216	1047	2126	16260
Bluefish								
Age 0	1341	514	119	0	113	19	9	2115
Age 1	1242	1750	271	0	1547	517	24	5351
Age 2	1683	4714	0	3937	1030	0	0	11360
Weakfish								
Age 0, *a*	257	0	0	5	0	0	34	296
Age 0, *b*	103	0	0	3	0	0	10	116
Age 0, *c*	48	0	0	3	0	0	3	54
Age 1, *a*	1323	736	0	138	172	159	234	2762
Age 1, *b*	638	355	0	66	83	76	113	1331
Age 2, *a*	183	2832	0	32	1962	433	210	5652
Age 2, *b*	100	1539	0	17	1066	235	114	3071

in the age-0 to age-2 classes. However for age-2 fish, menhaden consumption was greatest for bluefish. Age-2 weakfish from cohort *a* also consumed more menhaden than age-2 striped bass, but consumption of menhaden by age-2 cohort *b* weakfish was 18% lower than by age-2 striped bass (Fig. 6).

Trophic usage

Pooling annual diet information on the basis of the trophic pathway of each prey type (benthic or pelagic) showed different ontogenetic patterns in trophic usage across species (Fig. 10). The trophic source of prey for bluefish and weakfish showed similar ontogenetic patterns. Bluefish and weakfish trophic usage as assessed by annual diets showed that over 50% of all prey for age-0 through age-2 fish was from the pelagic realm. About 90% of the prey of age-0 bluefish and weakfish were species believed to be associated with pelagic food webs, but the use of prey associated with benthic food webs increased in age-1 (30–35% benthic) and age-2 fish (43% benthic). In contrast, the use of prey associated with benthic food webs by striped bass was greatest for age-0 (94%) and age-1 (44%) fish. Age-2 and older striped bass fed mainly on pelagic food web resources (68–75% pelagic), largely because of the importance of Atlantic menhaden to annual diets of older striped bass (Fig. 10).

Predator supply and demand

Predator demand (consumption potential according to fish size and temperature regime under field conditions) exceeded supply (estimated amount that was consumed under field temperature regimes and field estimates of growth) for all predator cohorts and the disparity between supply and demand increased with fish age. Because supply and demand are presented in terms of cumulative consumption (Figs. 11–14), supply is equal to demand when slopes are equal. Similarly, demand exceeds supply (surplus demand) when the demand curve is increasing faster than the supply curve. Prey supply for age-0 bluefish was close to demand throughout the year (Fig. 11). Prey supply was less than demand for age-0 weakfish and striped bass (Fig. 11). The difference between supply and demand appeared to increase after day 250 (early September) for age-0 striped bass and weakfish. Overall, demand for prey by age-0 striped bass exceeded supply by 160 g (67%) and for age-0 weakfish by 150 g (49%).

Predator demand for age-1 piscivores showed a seasonal pattern (Fig. 12). Striped bass demand for prey exceeded supply by day 90, but between day 150 and day 275 the slopes of the supply and demand curves were nearly parallel, indicating that supply and demand during this period were equal. A second period of prey shortage occurred after day 275 and continued through the end of the year for age-1 striped bass. Predator supply was less than demand throughout the year for age-1 weakfish and bluefish (Fig. 12). Predator demand exceeded supply by 61% for striped bass, 61% for bluefish, and 50% for weakfish.

Surplus demand by age-2 predators differed among the predator species. Bluefish demand was about equal to supply until day 200 (Fig. 13). After day 200, bluefish demand increased faster than supply. Total surplus demand by bluefish was 41%. Prey supply was equal to demand for age-2 striped bass through day 100, while water temperatures were still below 12°C. However, after day 100 the demand

Fig. 5. Cumulative consumption (g/fish) by age-0 striped bass (SB-0), bluefish (BF-0), and weakfish cohorts *a* (WF-0 A) and *b* (WF-0-B) during annual residency.

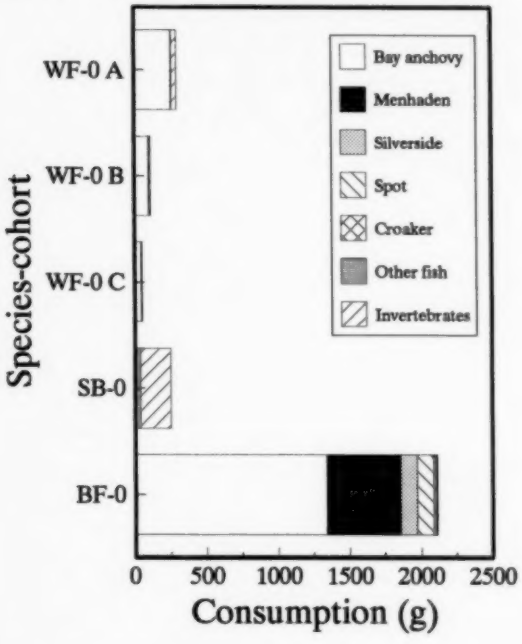
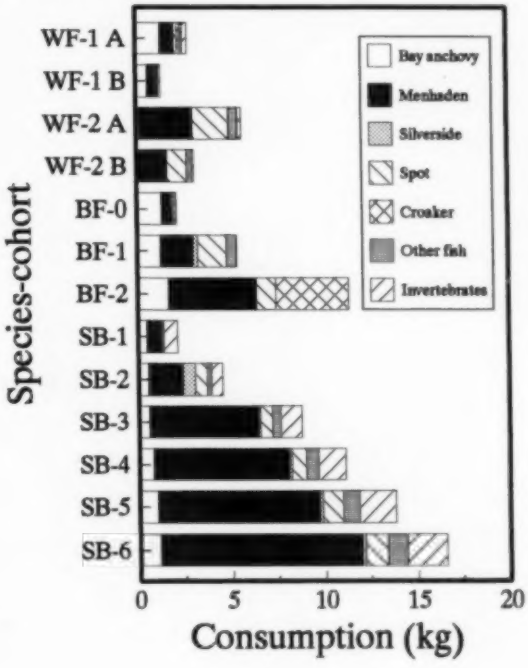


Fig. 6. Cumulative individual consumption (kg) by age-0 bluefish and age-1 and older striped bass, bluefish, and weakfish during estuarine residency. In the abbreviations, BF is bluefish, SB is striped bass, and WF is weakfish; numerals 0–6 represent the age-class; and the letters A and B represent cohorts for weakfish.



for prey by striped bass continued to increase slowly relative to prey supply until the end of the year when total demand exceeded supply by 95% (Fig. 13). Prey supply for age-2 weakfish was less than demand throughout the year (Fig. 13). By the end of the year, prey demand by age-2 weakfish was 78% higher than supply.

Prey demand by age-3 through age-6 striped bass was equal to supply early (days 1–60) and again late in the year (after day 330), but was less than supply for much of the year (Fig. 14). Total prey demand by age-3 striped bass exceeded supply by 80%, while demand by age-4 through age-6 striped bass was 101–103% higher than supply (Fig. 14).

Conversion efficiency

Annual conversion efficiency generally declined with age for striped bass, bluefish, and weakfish (Table 8). Striped bass annual conversion efficiency (cumulative growth (g)/ cumulative consumption (g)) was highest for age-0 (0.16) and age-1 (0.15) fish, but decreased to 0.10 to 0.02 for age-2 through age-6 fish. Annual conversion efficiency of weakfish was highest for age-0 fish (0.14–0.23 for cohorts *a* to *c*) and declined to 0.10–0.11 for age-1 and age-2 weakfish. Annual conversion efficiency of bluefish was 0.18 for age-0 fish, but declined to 0.09–0.12 for older cohorts (Table 8).

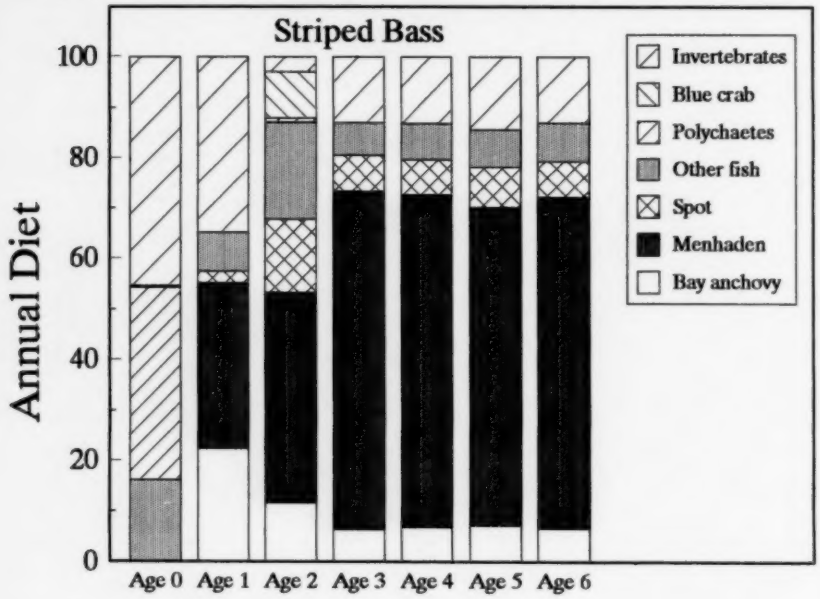
Population consumption

Consumption by the hypothetical predator populations varied across species and within species. Consumption by age-0 to age-2 bluefish was estimated at 27 167 t, about 2.7 times the population consumption of age-0 to age-6 striped bass and 5.4 times the age-0 to age-2 (cohort *a*) population consumption (Fig. 15). The high population consumption by bluefish is attributed to their rapid growth rate that increases survival with the weight-dependent mortality function, and also increases the consumption rate per individual. Population consumption by weakfish age 0 to age 2, cohort *a*, was higher than that of similar-aged striped bass despite a longer modeling period (365 vs. 154 d) for striped bass (Fig. 15).

Within a species the population-level consumption also varied by cohort. Bluefish consumption was similar among age-0 through age-2 cohorts, ranging from 8689 to 9470 t/year. Composition of bluefish consumption changed with ontogeny, with more menhaden and less anchovy eaten with increasing age (Fig. 16). Population-level consumption by striped bass was highest among the middle age classes and lowest for age-0 fish, but consumption by age-1 through age-5 striped bass was within 32% of age-3 cohort consumption (Fig. 16). As with bluefish, anchovy consumption declined and menhaden consumption increased as striped bass aged.

Consumption by the hypothetical weakfish population had the same ontogenetic pattern in the use of bay anchovy as for the other predators, but consumption also varied with cohort and age-class (Fig. 16). Although bay anchovy dominated the diets of age-0 weakfish and declined in importance to older fish, total consumption of anchovy by

Fig. 7. Annual diet composition (proportion of total annual consumption (g)) of age-0 through age-6 striped bass in the Chesapeake Bay.



age-1 weakfish was higher than for age-0 weakfish. Within an age-class the consumption by cohort *a* was always higher than that of cohort *b* or *c*, despite the same initial numbers in each cohort (Fig. 16).

Discussion

It is clear from modeling exercises that from a trophic perspective all piscivores in Chesapeake Bay are not created equal. As individuals, and within hypothetical populations, bluefish clearly have the greatest potential to affect prey populations and to compete with other predators for food either directly or through prey depletion. The hypothetical bluefish population was estimated to consume 27 000 t of prey of which about 17 000 t were either bay anchovy or Atlantic menhaden. During the portion of the year when bluefish resided in the bay we estimated that they consumed more anchovy and menhaden than the populations of age-0 through age-6 striped bass (annual residents) and age-0 through age-2 weakfish combined.

The potential for competition among piscivores may be greatest among bluefish and weakfish (all ages) or bluefish and older striped bass. Young striped bass appeared to trophically separate themselves from weakfish and bluefish by feeding on benthic sources as young fish, but as striped bass aged, Atlantic menhaden became increasingly important, with roughly 5000 t (50%) of the annual population consumption consisting of menhaden. Bluefish and weakfish trophic partitioning were similar to each other. In the population-level modeling, age-0 bluefish ate more bay anchovy (5000 t) than all weakfish cohorts combined (2200 t) despite the fact that anchovy more completely dominated weakfish diets.

Several prey species provide most of the energy required for the production of striped bass, bluefish, and weakfish in the Chesapeake Bay. Atlantic menhaden, bay anchovy, and spot represented 65–99% of total prey consumed by all age-classes of piscivores during estuarine residency. In general, bay anchovy were more important to the annual diets of smaller predators (e.g., age-0 weakfish and bluefish). As predators aged, their annual diets were made up increasingly of larger prey such as Atlantic menhaden and spot.

Atlantic menhaden, bay anchovy, and spot occupy different trophic positions in the Chesapeake Bay food web. Atlantic menhaden eat primarily phytoplankton (Jeffries 1975; Lewis and Peters 1984), bay anchovy eat mostly zooplankton (Klebasko 1991), and spot feed mainly on benthos (Homer and Boynton 1978). Therefore, both the abundance and age structure of piscivore populations may affect trophic energy flow in different ways as a result of species-specific and ontogenetic differences in trophic use.

Bluefish had the highest predatory impact of the piscivores. Bluefish grew faster and consumed more prey than other species of similar age or size. Bluefish was also the species that was able to come closest to balancing prey demand and supply, i.e., bluefish nearly realized their maximum consumption rates on an annual basis. However, these consumption rates may not estimate the total predatory mortality inflicted by bluefish upon prey communities. Bluefish have shearing dentation that allows them to disable prey too large to swallow whole (Baird 1873; Bigelow and Schroeder 1953). Anecdotal accounts from Chesapeake Bay fishermen also tell of feeding frenzies in which individual bluefish reportedly regurgitate and continue eating. The process of disabling prey or regurgitation may lead to higher predatory mortality on prey fish

Fig. 8. Annual diet composition (proportion of total annual consumption (g)) by age-0 through age-2 bluefish in the Chesapeake Bay.

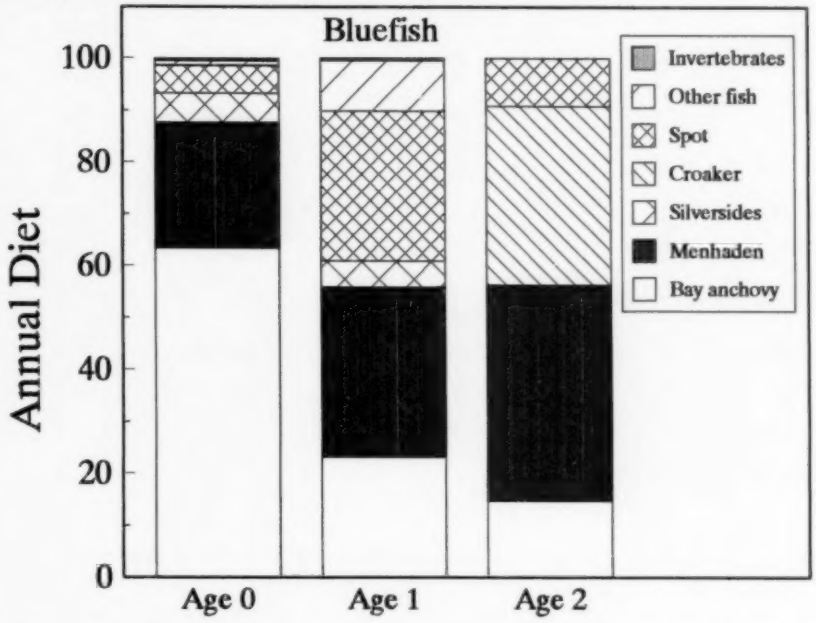
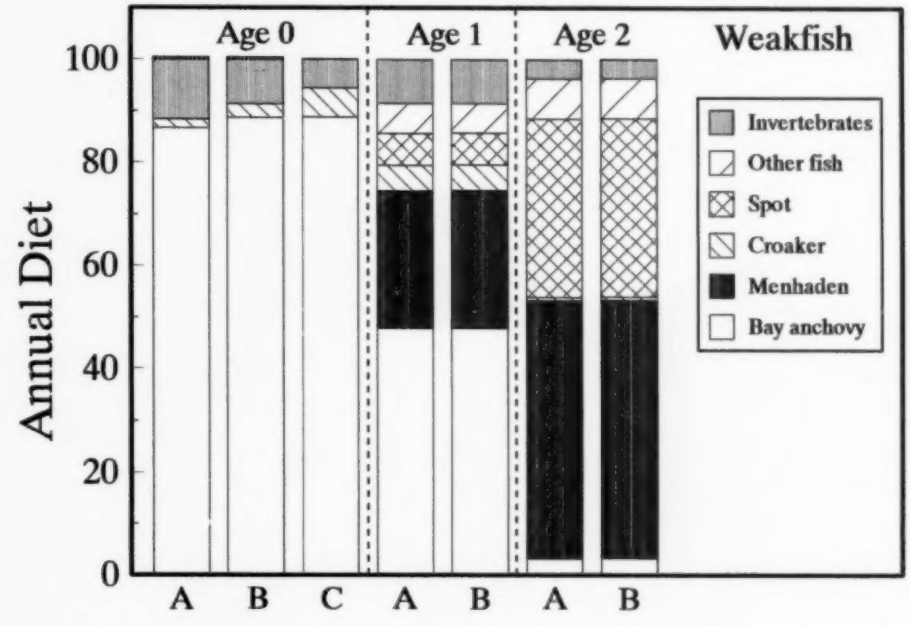


Fig. 9. Annual diet composition (proportion of total annual consumption (g)) by age-0 through age-2 weakfish in the Chesapeake Bay.



communities than is predicted by this analysis because the bioenergetics model only estimates the consumption required to meet growth and maintenance costs.

Estimates of consumption by predators allowed a measure of the relative supply of prey, or prey availability, to different piscivore species and cohorts. Bioenergetics simulations under the same initial conditions except with predators feeding at maximum rates (given temperature and fish size considerations) provided an estimate of the predatory demand for each fish. Our data for the relatively

Fig. 10. Annual use of prey associated with benthic food webs (such as spot, croaker, gammarids, and polychaetes) and prey associated with pelagic food webs (such as anchovy, menhaden, and silversides) by striped bass (ages 0–6), bluefish (ages 0–2), and weakfish (ages 0–2, cohorts *a*, *b*, *c*) in the Chesapeake Bay.

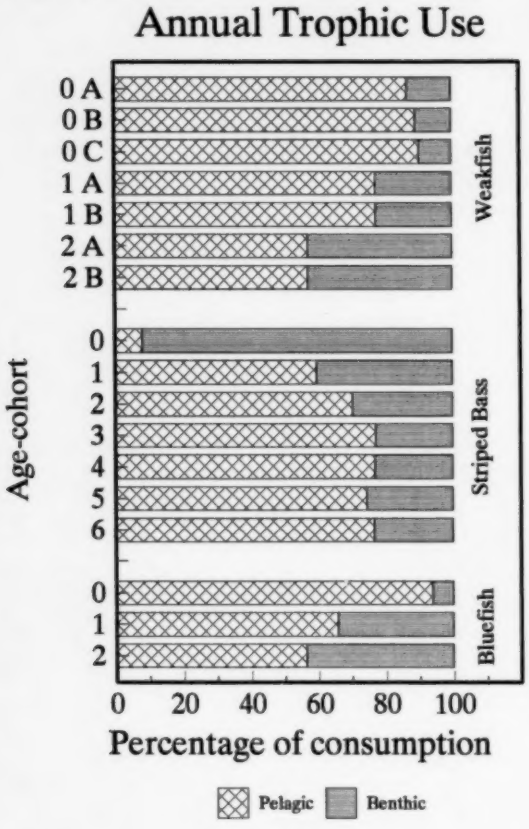
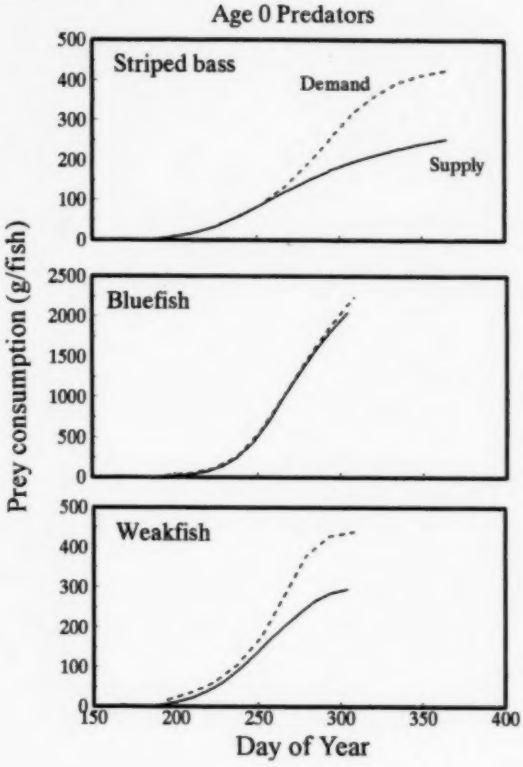


Fig. 11. Seasonal comparison of predator demand (unlimited food, temperature-driven C_{max}) and prey supply (actual consumption based on observed growth) for an individual age-0 striped bass, bluefish, and weakfish (cohort *a*) in the Chesapeake Bay.



short periods of the maximum consumption rate (C_{max}) experiments do not suggest any significant decline in consumption rate over time. Therefore, we assumed that fish will feed at C_{max} for extended periods. Comparison of the two curves (supply and demand) over time showed that consumption by striped bass, weakfish, and to a lesser degree bluefish, was limited by the supply of prey. Prey supply and predator demand of bluefish were closer to equality, but for age-1 bluefish prey supply still fell short of demand by 61%.

Because bluefish consumption was relatively close to their demand, bluefish appear to be the piscivores best suited to the Chesapeake Bay environment. Bluefish may also be the species of greatest potential concern for trophic competition with weakfish and striped bass. On broad spatial scales, the biomass of potential prey to the three piscivores is probably equal because all appear to be capable of feeding on the same prey species and sizes. However, possibly because of their high mobility (Olla and Studholme 1971), bluefish are able to consistently come closer to meeting their predatory demands than the other piscivores. Therefore, bluefish may have the highest potential to at least locally reduce prey and influence production and dynamics at lower trophic levels. We suggest that bluefish may in fact be reducing prey availability to the other piscivores in the Chesapeake Bay as was reported for bluefish in the northwest Atlantic Ocean (Safina and Burger 1989).

Although predatory demand exceeds supply for estuarine piscivores, it is difficult to determine if bottom-up or top-down effects tend to dominate as controlling mechanisms to limit piscivore production in the Chesapeake Bay. Predators are usually not feeding at maximum levels. Therefore, unless predators are not trying to maximize consumption, the availability of prey must at times be a limiting factor (we know that temperature is not generally the limit or else demand for prey would be met). Luo and Brandt (1993) measured the biomass of pelagic fishes in the Chesapeake Bay bimonthly from July 1989 through July 1990. Pelagic fish biomass (mostly bay anchovy and some Atlantic menhaden) was 5–15 $g \cdot m^{-2}$ between late July and December, but averaged less than 5 $g \cdot m^{-2}$ between January and mid-July (Luo and Brandt 1993). Estimates of production of

Fig. 12. Seasonal comparison of predator demand (unlimited food, temperature-driven C_{max}) and prey supply (consumption achieved based on observed growth) for an individual age-1 striped bass, bluefish, and weakfish (cohort *a*) in the Chesapeake Bay.

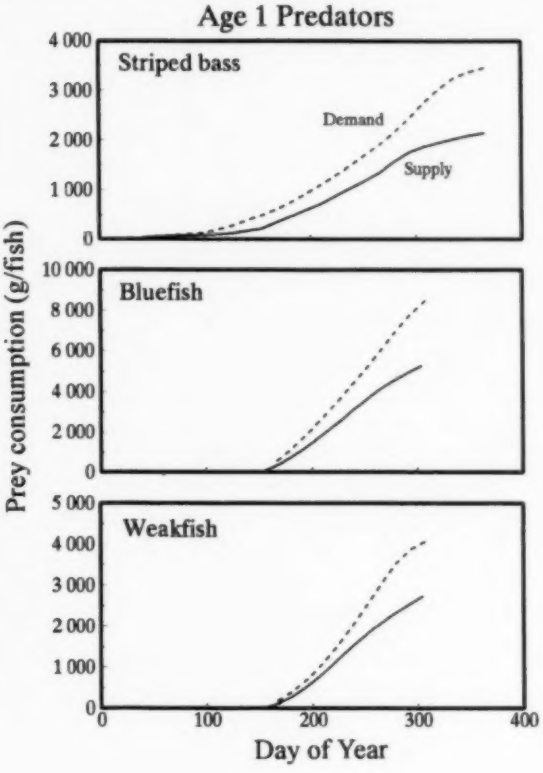
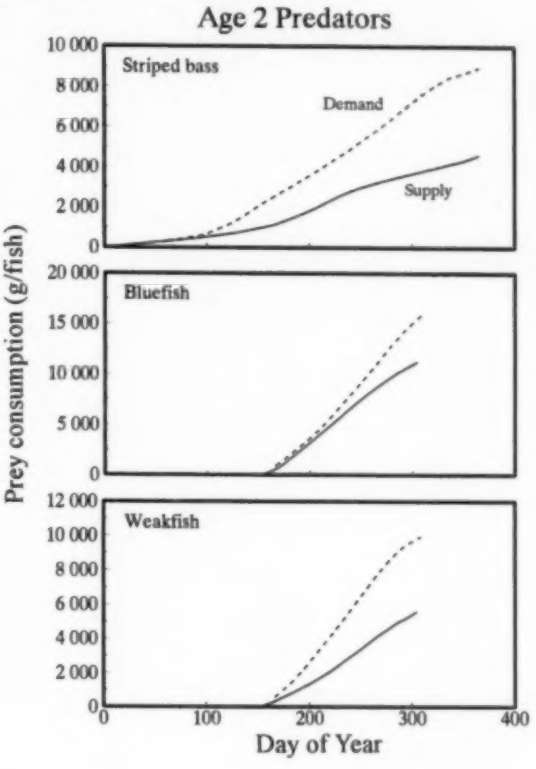


Fig. 13. Seasonal comparison of predator demand (unlimited food, temperature-driven C_{max}) and prey supply (consumption achieved based on observed growth) for an individual age-2 striped bass, bluefish, and weakfish (cohort *a*) in the Chesapeake Bay.



bay anchovy in the mid-Chesapeake Bay averaged 175 kg·km^{-2}·d^{-1} between day 226 (mid-August) and day 336 (November; Luo and Brandt 1993). Obviously, anchovy is not only a fuel of striped bass, bluefish, and weakfish. Other predators are known to feed on anchovy as well (Homer and Boynton 1978), but if the daily production of anchovy were available only to striped bass, bluefish, and weakfish, and these predators ate only anchovy, the daily production of anchovy could feed 10–11 age-6 striped bass, 15 age-2 bluefish, or 31 age-2 (cohort *a*) weakfish per square kilometre annually. Although they have not yet been quantified in Chesapeake Bay, other prey species such as Atlantic menhaden also have high production rates (Rippetoe 1993). Therefore, it seems that the biomass of prey should not be limiting consumption by piscivores. However, the January through July period of low pelagic prey fish abundance (Luo and Brandt 1993) coincides with the period when demand most exceeds supply for most estuarine predators. For striped bass, supply was about equal to demand during the first several months of the year. During this time water temperatures are low (<10°C) and consumptive demands are limited by low temperatures. Between April and July water temperatures are higher, pelagic fish biomass is low (Luo and Brandt 1993), and apparently consumption is limited by the availability of prey.

Bluefish and weakfish residence in the Chesapeake Bay coincides with the period of highest pelagic prey biomass. We would expect that prey supply would be highest during the period of high prey biomass and thus the chances of predator demand exceeding supply would be lower at this time. For bluefish (except age-1) this was the case, but for age-0 through age-2 weakfish, demand exceeded supply between day 200 and day 250. Age-0 and age-1 weakfish are feeding on bay anchovy during this time and anchovy biomass should be at a peak during this time (Newberger 1989; Luo and Brandt 1993). Given dietary history and prey abundance it would be expected that demand for prey should be met by weakfish at this time of year. It could be that consumption and growth by weakfish are limited more by the availability of prey owing to spatial heterogeneity of prey, prey behavior, and environmental conditions (e.g., turbidity) rather than simply by the abundance of prey.

Clearly the issue of top-down versus bottom-up control in Chesapeake Bay is a complex one. In all likelihood each

Fig. 14. Seasonal comparison of predator demand (unlimited food, temperature-driven C_{max}) and prey supply (consumption achieved based on observed growth) for an individual age-3 through age-6 striped bass in the Chesapeake Bay.

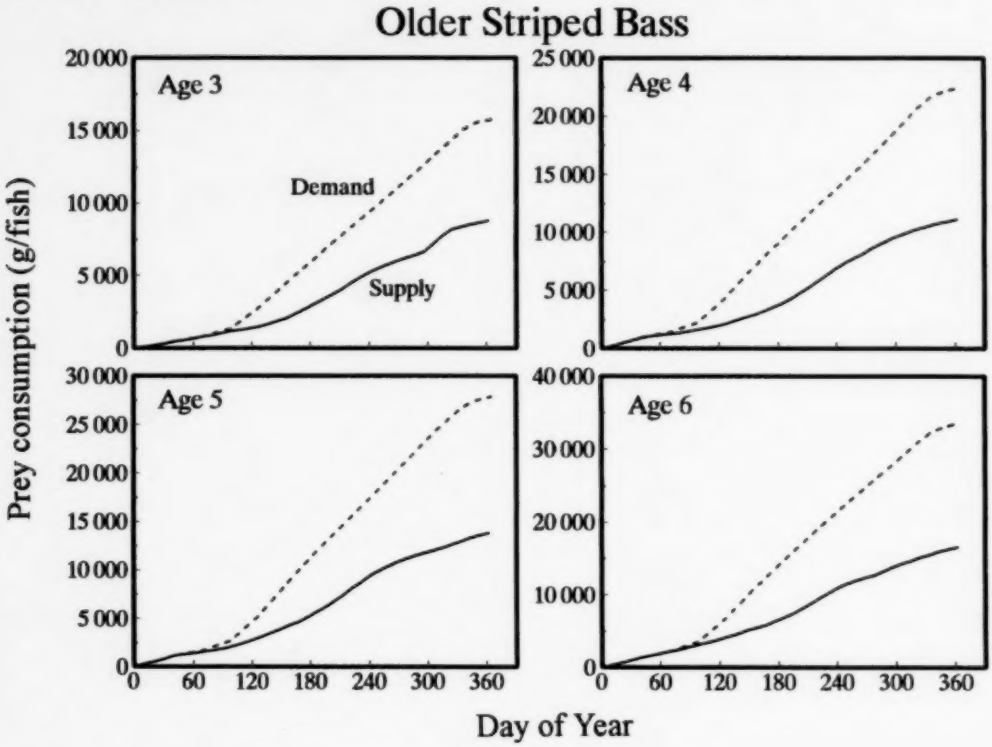


Fig. 15. Relative consumption by hypothetical populations of striped bass, bluefish, and weakfish in the Chesapeake Bay. Hypothetical populations are based upon a weight-dependent mortality function and constant annual recruitment of 10 million (1 g) age-0 fish.

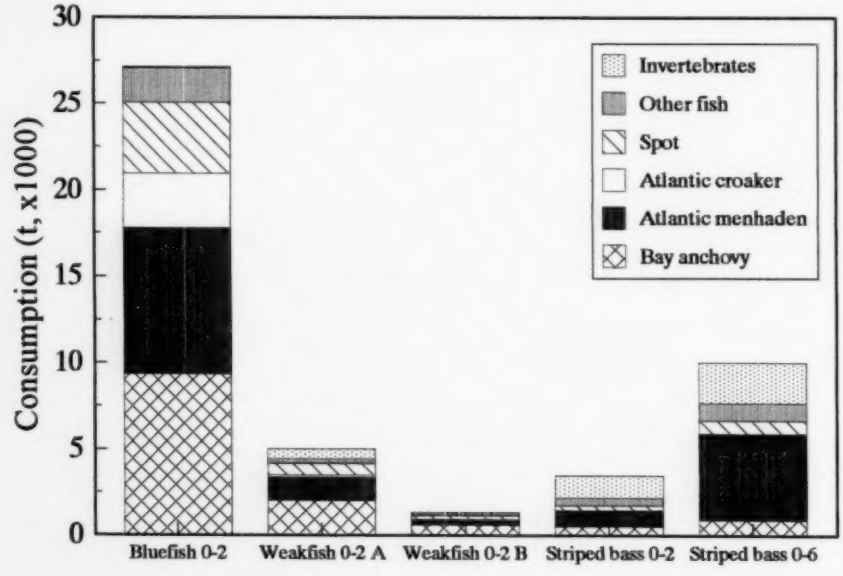


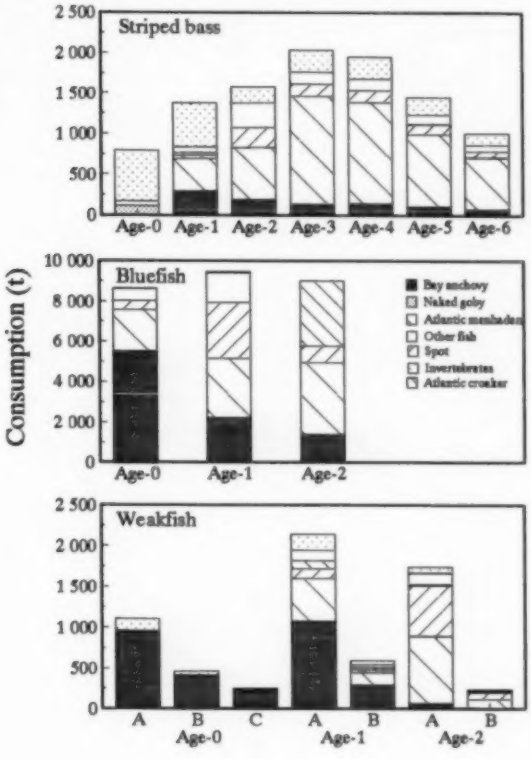
Table 8. Annual conversion efficiencies (GCE = change in biomass (g)/annual consumption (g)) of age-0 to age-2 weakfish and bluefish and age-0 through age-6 striped bass in Chesapeake Bay.

Cohort	Biomass (g)	GCE
Striped bass		
Age 0	30	0.16
Age 1	285	0.15
Age 2	399	0.09
Age 3	843	0.10
Age 4	463	0.04
Age 5	331	0.02
Age 6	442	0.03
Weakfish		
Age 0, *a*	42	0.14
Age 0, *b*	20	0.17
Age 0, *c*	12	0.23
Age 1, *a*	311	0.11
Age 1, *b*	135	0.10
Age 2, *a*	640	0.11
Age 2, *b*	341	0.11
Bluefish		
Age 0	372	0.18
Age 1	477	0.09
Age 2	1361	0.12

plays a role in regulating fish production in the Chesapeake Bay. The finding that demand for prey exceeds supply during some times of the year could support either hypothesis; however, for striped bass the timing of this event coincides with low prey availability. Unfortunately, this still does not clarify the issue of whether top-down or bottom-up forces dominate even on short time scales because it is still unclear whether supply was low due to predator-induced changes (top down) or low prey production (bottom up). In the case of bay anchovy, production estimates are available that suggest that the highest annual production in Chesapeake Bay occurs in bay anchovy larval stages between July and September (Wang 1992), but other analyses have suggested peak production in post-larvae between September and November (Newberger 1989; Luo and Brandt 1993). Most production during May–July goes into reproduction and recruitment of young anchovy, two sources that are at least temporally unavailable to striped bass, bluefish, and weakfish. Information on the production and abundance of other prey available to piscivores is lacking in Chesapeake Bay. However, unless the production of other prey fills the May–July gap in available prey fish production, the possibility exists for top-down control to occur at least seasonally in the Chesapeake Bay.

In general, young predators came closer to balancing prey supply and demand than older age-classes. We found that young predators (except age-0 striped bass) fed heavily on bay anchovy at the time when bay anchovy production was most rapid (Luo and Brandt 1993). Bay anchovy are the most abundant pelagic fish in the Chesapeake Bay (Horwitz 1987; Brandt 1990), but perhaps because of their small

Fig. 16. Cohort-specific consumption within the hypothetical populations of striped bass, bluefish, and weakfish.



size, they were not consumed much by age-2 and older piscivores. Surprisingly, while the abundant bay anchovy was not being consumed by striped bass, predator demand by older striped bass cohorts exceeded supply by as much as 103%.

The high surplus demand of predators and low use of the most abundant prey species suggest that the Chesapeake Bay may be better suited as a nursery area for young piscivores than as a production area for older fish. Scope for growth of 1000-g striped bass, bluefish, and weakfish (Hartman and Brandt 1995*c*) indicated that high summer water temperatures were not the factor limiting growth of older piscivores in the Chesapeake Bay. Under ad libitum feeding, growth of about 2% of body weight·d^{-1} was possible for 1000-g fish of each species throughout the range of summer temperatures (20–29°C) typically observed in the Chesapeake Bay. We also observed positive growth of over 1%·d^{-1} for several 2.5-kg striped bass at 29°C during C_{max} experiments.

The behavior of adult striped bass (2500–3100 g) at 29–30°C in the laboratory did not suggest stress. Adult striped bass activity was similar to that at 15–25°C with low activity except during feeding. If adult striped bass were not stressed by temperatures of 25–30°C and positive growth is possible for migratory-age striped bass during summer in the Chesapeake Bay, then perhaps prey supply and

availability of (larger) suitable prey, rather than temperatures that cannot support growth (Coutant and Benson 1990), may account for the migration of older striped bass to coastal waters.

The period of the year when predatory demand exceeded prey supply varied with species and cohorts, with no clear species- or age-related correlation. Most predators did exhibit a shortage of prey during summer or fall. The shortfall between predatory demand and prey supply for striped bass was typically higher than for like-aged weakfish or bluefish. Striped bass predatory demand was over 80% higher than prey supply for older (age-4 to age-6) fish. This suggests that conditions may not be as favorable for striped bass growth as for growth of bluefish or weakfish, or that growth conditions are now less favorable than they once were in the Chesapeake Bay.

There is little evidence to support lower growth of striped bass now in comparison to earlier time periods in the Chesapeake Bay. Comparisons of the average size-at-age of striped bass from recent years with those from 1957 showed that growth of striped bass is similar to that reported in earlier years. Mansuetti (1961) reported that age-2 striped bass weighed between 312 g (males) and 369 g (females). In this study, age-2 fish averaged 315 g. Size-at-age of older fish was also similar between 1957 and the present study, although variability in size was large in both studies (Mansuetti 1961). However, the similarities in growth rates and variability from 1957 to the present suggest that despite numerous changes in the bay ecosystem during the last 35 years, conditions for growth of striped bass are similar or striped bass have adapted to the changes.

One of the advantages of bioenergetics models is that they allow construction of annual diets on the basis of the annual contribution of prey to predator production. These annual diets give a much clearer picture of the relative importance of various prey or trophic sources than the more common methods of reporting diets on the basis of percent biomass, frequency of occurrence, or other indices. Annual diets based on bioenergetics integrate growth, temperature, and prey quality to arrive at more accurate reflections of the contributions of prey to fish production. We used the bioenergetics models developed in this study to answer the question regarding the relative roles of predators in the Chesapeake Bay and to quantify the amount and trophic source of prey required to fuel piscivore production.

Interesting trends in resource use were apparent across species. Striped bass increased their use of pelagic food web resources as they aged, whereas weakfish and bluefish increased their use of benthic food web resources with ontogeny. All predators switched to larger prey species as they aged, primarily menhaden for striped bass, but a combination of menhaden, spot, and croaker for older bluefish and weakfish. The increased use of prey from benthic food webs by older weakfish and bluefish may be in response to reduced profitability in feeding on the smaller fish such as bay anchovy (Werner and Hall 1974; Townsend and Hughes 1981; Werner and Mittlebach 1981). Alternatively, increased use of benthic prey could also be due to increasing relative availability of larger prey from benthic sources, and perhaps to vertical displacement owing to hypoxia, rather than a change in feeding mode from pelagic to benthic.

The trophic use patterns of the three predators may have important consequences for the future of these fish in the Chesapeake Bay. In a food web analysis of Chesapeake Bay, Baird and Ulanowicz (1989) suggested that piscivorous species deriving the majority of their energy via benthic sources may be favored in the bay because of poor trophic efficiencies in pelagic pathways because of gelatinous predators. These gelatinous predators feed at several trophic positions but have no real link to piscivore production and thus are a net sink of energy to piscivore production. If such generalizations continue to hold true, our analyses indicate that striped bass would find the bay less favorable for production with increasing age, while bluefish and weakfish would find more favorable conditions for production with ontogeny.

The increased use of menhaden by older piscivore cohorts, in particular striped bass, may be of political importance. Atlantic menhaden are not only consumed by piscivorous fish, but are also a resource species, setting up a possible competitive situation. Our analyses found low food availability for age-3 and older striped bass during spring and early summer when yearling and older prey fish would dominate the diets. Atlantic menhaden dominate the fall through spring diets of older striped bass (Hartman and Brandt 1995*a*) and would be expected to contribute to diets in May through June if they were available. However, a large commercial fishery for menhaden exists in Virginia and North Carolina and much of the emigrating age-0 menhaden stock is believed to be intercepted by this fishery, thus reducing the numbers that would return to the middle to upper Chesapeake Bay during the following spring (Arenholz et al. 1987). The Atlantic coast harvest of Atlantic menhaden was 428 198 t in 1990, most of which (92%) came from the reporting zone encompassing the Virginian and North Carolinan fisheries for menhaden (Food and Agriculture Organization of the United Nations 1992). Bioenergetically derived consumption estimates showed that an age-6 striped bass or an age-2 bluefish would eat 10 610 and 4714 g of menhaden·yr^{-1}, respectively. On the basis of these consumption estimates it would take 40.4 million age-6 striped bass or 90.8 million age-2 bluefish to equal the 1990 east coast U.S. harvest of Atlantic menhaden. Thus, because the actual numbers of striped bass and bluefish must be much lower than those above, predation by piscivores upon menhaden seems small relative to commercial harvest of menhaden.

The Atlantic menhaden fishery has been diagnosed as suffering from growth overfishing (Vaughan and Merriner 1991) and several plans were set forth by the Atlantic States Marine Fishery Commission to conserve menhaden (Vaughan and Merriner 1991; Vaughan and Smith 1991). These management options involved reducing effort or closing nearshore areas to fishing in an effort to reduce the harvest of young age-classes of menhaden. In all likelihood any management measures that permit increased escapement and presumably increased migration of age-1 and older menhaden to the Chesapeake Bay will benefit the production of striped bass, bluefish, and weakfish. Analysis of supply and demand for most age-2 and older

piscivores indicated a shortage of prey supply during spring and early summer. Increased escapement of age-0 menhaden should help to provide additional prey supply during this period and increase the production and annual conversion efficiencies for piscivores in the bay. However, the specific increase in production cannot be directly predicted at this time.

The absolute role of striped bass, bluefish, and weakfish as predators in the Chesapeake Bay must await further information on the population dynamics of these species. We estimated the consumption by hypothetical predator populations on the basis of 10 million recruits and constant recruitment. These modeling analyses provided insights into the relative predatory impact of different predator cohorts and also suggested that bluefish had the highest predatory demand per recruit of the three species. However, total consumption estimates for these species would require estimates of the abundances and mortality rates of each life stage. Hydroacoustics may be a possible tool in estimating population numbers in the bay particularly for striped bass, but estimates for bluefish and weakfish would be more difficult because of the mobile and demersal behaviors of the latter two fish. More detailed mortality rate estimates are required even to place consumption into relative terms. Depending upon the choice of mortality rate for age-0 fish the population level consumption by striped bass, bluefish, and weakfish could be altered from mostly benthic prey (striped bass) to mostly menhaden, or mostly anchovy or other species (bluefish). Because mortality is a sensitive parameter with respect to our population-level modeling, such changes greatly change our derived perception of energy flow to piscivores in the system, and more reliable estimates of young through adult mortality rates are needed for fish in the Chesapeake Bay.

The bioenergetics models developed here should provide a framework for ecosystem-level studies of the Chesapeake Bay. At such time as population size estimates or mortality rate information become available, a more detailed analysis of Chesapeake Bay trophic interactions will become possible through the use of the bioenergetics models developed and validated in this study.

Acknowledgements

The authors thank W. Boynton, E. Houde, J. Mihursky, A. Popper, and R. Ulanowicz for their reviews of earlier versions of the manuscript and H. Millsaps for providing us with water temperature data. Support for this study was provided by the Electric Power Research Institute's Fellowship Program in Population Biology (EPRI-09-07), the Sport Fishing Research Institute (SERP-90-21), the Maryland Sea Grant Program (NA90AA-D-SG063R/F-65), and the National Science Foundation's Biological Oceanography Program (OCE-9116071 and OCE-9415740).

References

Arenholz, D.W., W.R. Nelson, and S.P. Epperly. 1987. Population and fishery characteristics of Atlantic menhaden, *Brevoortia tyrannus*. Fish. Bull. 85: 569–600.

Atlantic States Marine Fisheries Commission. 1992. Weakfish fishery management plan amendment 1. Fisheries Management Rep. No. 20, Atlantic States Marine Fisheries Commission, Dover, Del.

Baird, S.F. 1873. Natural history of some of the more important food fishes of the south shore of New England. II. The bluefish (*Pomatomus saltatrix*) (Linn.). *In* Report of the U.S. Fisheries Commission for 1871–1872. U.S. Fisheries Commission, Washington, D.C. pp. 235–252.

Baird, D., and R.E. Ulanowicz. 1989. The seasonal dynamics of the Chesapeake Bay ecosystem. Ecol. Monogr. 59: 329–364.

Barber, M.C., L.A. Suarez, and R.L. Lassiter. 1991. Modelling bioaccumulation of organic pollutants in fish with an application to PCB's in Lake Ontario salmonids. Can. J. Fish. Aquat. Sci. 48: 318–337.

Bartell, S.M., J.E. Breck, R.H. Gardner, and A.L. Brenkert. 1986. Individual parameter perturbation and error analysis of fish bioenergetics models. Can. J. Fish. Aquat. Sci. 43: 160–168.

Beauchamp, D.A., D.J. Stewart, and G.L. Thomas. 1989. Corroboration of a bioenergetics model for sockeye salmon. Trans. Am. Fish. Soc. 118: 597–607.

Bigelow, H.B., and W.C. Schroeder. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildl. Serv. Fish. Bull. No. 53.

Borgmann, U., and D.M. Whittle. 1992. Bioenergetics of PCB, DDE, and mercury dynamics in Lake Ontario lake trout (*Salvelinus namaycush*): a model based on surveillance data. Can. J. Fish. Aquat. Sci. 49: 1086–1096.

Boudreau, P.R., and L.M. Dickie. 1989. Biological model of production based on physiological and ecological scaling of body size. Can. J. Fish. Aquat. Sci. 46: 614–623.

Brandt, S.B. 1990. Acoustic quantification of fish abundance in the Chesapeake Bay. Progress report to Maryland Department of Natural Resources, Power Plant Research Program, Annapolis, Md. CBL Ref. No. [UMCEES]CBL 90-047.

Brandt, S.B., E.V. Patrick, D.M. Mason, K.J. Hartman, and M.J. Klebasko. 1991. Bioacoustics and bioenergetics: trophic supply and demand in the Chesapeake Bay. *In* New perspectives in the Chesapeake system: a research and management partnership. *Edited by* J.A. Mihursky and A. Chaney. Publ. No. 137, Chesapeake Research Consortium, Solomons, Md.

Brandt, S.B., D.M. Mason, and E.V. Patrick. 1992. Spatially explicit models of fish growth rate. Fisheries, 17: 23–35.

Carpenter, S.R., J.F. Kitchell, and J.R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience, 35: 634–639.

Carpenter, S.R., J.F. Kitchell, J.R. Hodgson, P.A. Cochran, M.M. Elser, D.M. Lodge, and D. Kitchner. 1987. Regulation of lake primary productivity by food web structure. Ecology, 68: 1863–1876.

Chadwick, H.K. 1968. Mortality rates in the California striped bass population. Calif. Fish Game, 54: 228–246.

Cochran, P.A., and K.J. Knutsen. 1988. Error in estimation of feeding rates from changes in mean body mass. Can. J. Fish. Aquat. Sci. 45: 1494–1498.

Cochran, P.A., and J.A. Rice. 1982. A comparison of bioenergetics and direct field estimates of cumulative seasonal food consumption by largemouth bass (*Micropterus salmoides*). *In* Gutshop '81: fish food habits studies. *Edited by* G. Cailliet and C. Simenstad. Washington Sea Grant, Seattle, Wash. pp. 88–96.

Connolly, J.P., and R. Tonelli. 1985. Modelling kepone in the striped bass food chain of the James River estuary. Estuarine Coastal Shelf Sci. 20: 349–366.

Coutant, C.C., and D.L. Bensen. 1990. Summer decline in habitat suitability for striped bass in Chesapeake Bay: reflections on a population decline. Trans. Am. Fish. Soc. 119: 757–778.

Cummins, K.W., and J.C. Wuycheck. 1971. Caloric equivalents for investigations in ecological energetics. Mitt. Int. Ver. Theor. Angew. Limnol. No. 18.

Darnell, R.M. 1958. Food habits of fishes and larger invertebrates of Lake Pontchartrain, Louisiana, an estuarine community. Tex. Univ. Mar. Sci. Publ. 5: 353–416.

Dey, W.P. 1981. Mortality and growth of young-of-the-year striped bass in the Hudson River Estuary. Trans. Am. Fish. Soc. 110: 151–157.

Dorazio, R.M., B.M. Florence, and C.M. Wooley. 1991. Stocking of hatchery-reared striped bass in the Patuxent River, Maryland: survival, relative abundance, and cost-effectiveness. N. Am. J. Fish. Manage. 11: 435–442.

Food and Agricultural Organization of the United Nations. 1992. Fishery statistics: catches and landings, 1990. Vol. 70. Food and Agricultural Organization of the United Nations, Rome, Italy.

Goshorn, D. 1993. Methodology for estimating year class strength from Chesapeake Bay pound net data. *In* Striped bass study annual workshop 1993. National Oceanic and Atmospheric Administration, Silversprings, Md. pp. 34–38. (Abstr.)

Goyke, A.P., and S.B. Brandt. 1993. Spatial models of salmonid growth rates in Lake Ontario. Trans. Am. Fish. Soc. 122: 870–883.

Hartman, K.J. 1993. Striped bass, bluefish, and weakfish in the Chesapeake Bay: energetics, trophic linkages, and bioenergetics model applications. Ph.D. dissertation, The University of Maryland System, College Park, Md.

Hartman, K.J., and S.B. Brandt. 1993. Systematic sources of bias in a bioenergetics model: examples for age-0 striped bass. Trans. Am. Fish. Soc. 122: 912–926.

Hartman, K.J., and S.B. Brandt. 1995*a*. Trophic resource partitioning, diets, and growth of sympatric estuarine predators. Trans. Am. Fish. Soc. 124: 520–537.

Hartman, K.J., and S.B. Brandt. 1995*b*. Estimating energy density of fish. Trans. Am. Fish. Soc. 124: 347–355.

Hartman, K.J., and S.B. Brandt. 1995*c*. Comparative energetics and the development of bioenergetics models for sympatric estuarine piscivores. Can. J. Fish. Aquat. Sci. 52: 1647–1666.

Hartman, K.J., and F.J. Margraf. 1992. Effects of prey and predator abundances on prey consumption and growth of walleyes in western Lake Erie. Trans. Am. Fish. Soc. 121: 245–260.

Hartman, K.J., and F.J. Margraf. 1993. Evidence of predatory control of yellow perch (*Perca flavescens*) recruitment in Lake Erie, U.S.A. J. Fish Biol. 43: 109–119.

Hewett, S.W. 1989. Ecological applications of bioenergetics models. Am. Fish. Soc. Spec. Symp. 6: 113–120.

Hewett, S.W., and B.L. Johnson. 1992. A generalized bioenergetics model of fish growth for microcomputers, version 2.0. University of Wisconsin Sea Grant Institute, Madison, Wis.

Hixon, J.H., and K.L. Heck, Jr. 1978. Feeding habits of fishes collected in the vicinity of Calvert Cliffs Nuclear Power Plant, 1971–1973 and 1975. Ref. No. 78-5, Benedict Estuarine Research Laboratories, Benedict, Md.

Homer, M., and W.R. Boynton. 1978. Stomach analysis of fish collected in the Calvert Cliffs region, Chesapeake Bay, 1977. Final report to Maryland State Department of Natural Resources, PPS Progress, Annapolis, Md. Ref. No. UMCEES 78-154.

Horwitz, R.J. 1987. Fish. *In* Ecological studies in the middle reach of Chesapeake Bay. Lecture notes on coastal and estuarine studies. No. 23. *Edited by* K.L. Heck. Springer-Verlag, Berlin. pp. 167–224.

Jeffries, H.P. 1975. Diets of juvenile Atlantic menhaden (*Brevoortia tyrannus*) in three estuarine habitats as determined from fatty acid composition of gut contents. J. Fish. Res. Board Can. 32: 587–592.

Kitchell, J.F, and J.E. Breck. 1980. Bioenergetics model and foraging hypothesis for sea lamprey (*Petromyzon marinus*). Can. J. Fish. Aquat. Sci. 37: 2159–2168.

Kitchell, J.F., D.J. Stewart, and D. Weininger. 1977. Application of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 34: 1922–1935.

Klebasko, M.J. 1991. Feeding ecology and daily ration of bay anchovy (*Anchoa mitchilli*) in the mid-Chesapeake Bay. M.S. thesis, University of Maryland, College Park, Md.

LaBar, G.W. 1993. Using bioenergetics models to predict the impact of increased lake trout predation on rainbow smelt following sea lamprey control. Trans. Am. Fish. Soc. 122: 942–950.

Lewis, V.P., and D.S. Peters. 1984. Menhaden: a single step from vascular plant to fishery harvest. J. Exp. Mar. Biol. Ecol. 84: 95–100.

Logan, D.T. 1985. Environmental variation and striped bass population dynamics: a size-dependent mortality model. Estuaries, 8: 28–38.

Luo, J., and S.B. Brandt. 1993. Bay anchovy *Anchoa mitchilli* production and consumption in mid-Chesapeake Bay based on a bioenergetics model and acoustic measures of fish abundance. Mar. Ecol. Prog. Ser. 81: 223–236.

Lyons, J., and J.J. Magnuson. 1987. Effects of walleye predation on the population dynamics of small littoral-zone fishes in a northern Wisconsin lake. Trans. Am. Fish. Soc. 116: 29–39.

Mansuetti, R.J. 1961. Age, growth, and movements of the striped bass, *Roccus saxatilis*, taken in size selective fishing gear in Maryland. Chesapeake Sci. 2: 9–36.

Maryland Department of Natural Resources. 1989. Investigation of striped bass in Chesapeake Bay 1988–1989. *In* Annual progress report. USFWS federal aid project F-42-R-2. Maryland Department of Natural Resources, Annapolis, Md.

Mercer, L.P. 1983. A biological and fisheries profile of weakfish, *Cynoscion regalis*. Spec. Sci. Rep. No. 39. North Carolina Department of Natural Resources and Community Development, Division of Fisheries, Moorehead City, N.C.

Mid-Atlantic Fishery Management Council. 1984. Striped bass fishery management plan. Mid-Atlantic Fishery Management Council, Washington, D.C.

Mid-Atlantic Fishery Management Council, Atlantic States Marine Fisheries Commission, National Marine Fisheries Service, New England Fishery Management Council, and South Atlantic Fishery Management Council. 1989. Fishery management plan for the bluefish fishery. Mid-Atlantic Fishery Management Council, Dover, Del.

Minton, J.W. and R.B. McLean. 1982. Measurements of growth and consumption of sauger (*Stizostedion canadense*): implication for fish energetics studies. Can. J. Fish. Aquat. Sci. 39: 1396–1403.

Newberger, T.A. 1989. Relative abundance, age, growth and mortality of bay anchovy in the mid-Chesapeake Bay. M.S. thesis, University of Maryland, College Park, Md.

Ney, J.J. 1990. Trophic economics in fisheries: assessment of demand–supply relationships between predators and prey. Aquat. Sci. 2: 55-81.

Norstrom, R.J., A.E. McKinnon, and A.S.W. De Frietas. 1976. A bioenergetics-based model for pollutant accumulation by fish: simulation of PCB and methylmercury residue levels in Ottawa River yellow perch (*Perca flavescens*). J. Fish. Res. Board Can. 33: 248–267.

Olla, B.L., and A.L. Studholme. 1971. The effects of temperature on the activity of bluefish, *Pomatomus saltatrix* L. Biol. Bull. (Woods Hole), 141: 337–349.

Rice, J.A., and P.A. Cochran. 1984. Independent evaluation of a bioenergetics model for largemouth bass. Ecology, 65: 732–739.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

Rippetoe, T.H. 1993. Production and energetics of Atlantic menhaden in Chesapeake Bay. M.S. thesis, University of Maryland System, College Park, Md.

Safina, C., and J. Burger. 1989. Population interactions among free-living bluefish and prey fish in an ocean environment. Oecologia, 79: 91–95.

Setzler, E.M., W.R. Boynton, K.V. Wood, H.H. Zion, L. Lubbers, N.K. Mountford, P. Frere, L. Tucker, and J.A. Mihursky. 1980. Synopsis of biological data on striped bass, *Morone saxatilis* (Walbaum). NOAA Tech. Rep. NMFS Circ. No. 433. FAO Synopsis No. 121.

Stewart, D.J., and M. Ibarra. 1991. Predation and production by salmonine fishes in Lake Michigan, 1978–88. Can. J. Fish. Aquat. Sci. 48: 909–922.

Stewart, D.J., J.F. Kitchell, and L.B. Crowder. 1981. Forage fish and their salmonid predators in Lake Michigan. Trans. Am. Fish. Soc. 110: 751–763.

Stewart, D.J., D. Weininger, D.V. Rottiers, and T.A. Edsall. 1983. An energetics model for lake trout, *Salvelinus namaycush*: application to the Lake Michigan population. Can. J. Fish. Aquat. Sci. 40: 681–698.

Szedlmayer, S.T., M.P. Weinstein, and J.A. Musick. 1990. Differential growth among cohorts of age-0 weakfish *Cynoscion regalis* in Chesapeake Bay. Fish. Bull. 88: 745–752.

Townsend, C.R., and R.N. Hughes. 1981. Maximizing net energy returns from foraging. *In* Physiological ecology: an evolutionary approach to resource use. *Edited by* C.R. Townsend and P. Calow. Blackwell, Oxford. pp. 86–108.

Vaughan, D.S., and J.V. Merriner. 1991. Assessment and management of Atlantic and Gulf menhaden stocks. Mar. Fish. Rev. 53: 49–57.

Vaughan, D.S., and J.W. Smith. 1991. Biological analysis of two management options for the Atlantic menhaden fishery. Mar. Fish. Rev. 53: 58–66.

Wahl, D.H., and R.A Stein. 1991. Food consumption and growth of three esocids: field tests of a bioenergetics model. Trans. Am. Fish. Soc. 120: 230–246.

Wang, S. 1992. Abundance, relative biomass, production and energy storage of bay anchovy, *Anchoa mitchilli*, in the Chesapeake Bay. M.S. thesis, University of Maryland, College Park, Md.

Werner, E.E., and D.J. Hall. 1974. Optimal foraging and size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). Ecology, 55: 1042–1065.

Werner, E.E., and G.G. Mittlebach. 1981. Optimal foraging: field tests of diet choice and habitat switching. Am. Zool. 21: 813–822.

Mitochondrial DNA divergence among populations of American shad (*Alosa sapidissima*): how much variation is enough for mixed-stock analysis?

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Abstract: We investigated the geographic distribution of the mitochondrial DNA (mtDNA) diversity of American shad from 15 North American rivers in 1992 with the intent of assessing sampling efficiency for future mixed-stock analysis. We observed 116 haplotypes among the 988 individuals assayed. Because no single or group of haplotypes completely discriminated river stocks or regional complexes, we investigated haplotype frequencies as stock descriptors. Analysis of four unique indices of haplotype divergence indicated that including rather than suppressing restriction site heteroplasmy increased resolution; however, the final results were not overwhelmingly dependent on this choice. A redundancy of variation among restriction enzymes diminished information returns rapidly after considering the six best enzymes, caused by physical linkage of restriction sites on the mtDNA molecule. Stock discriminatory power was tested by computing allocation efficiencies of mtDNA characters. When each individual was temporarily removed from the data set and reallocated to the various candidate populations on the basis of haplotypic similarity, 28% of the reallocations were correct, a fourfold increase over random success. We demonstrate that although the specific stock identity of individuals cannot be confidently established, the haplotypic arrays from baseline stocks can support stock identification and mixed-stock analysis for shad because rivers support stock-specific haplotype frequencies.

Résumé : En 1992, nous avons étudié la distribution géographique de la diversité de l'ADN mitochondrial de l'alose savoureuse de 15 rivières de l'Amérique du Nord afin de déterminer quelle devrait être l'efficacité de l'échantillonnage pour l'analyse ultérieure des stocks mixtes. Nous avons dénombré 116 haplotypes chez les 988 sujets évalués. Comme aucun haplotype ou groupe d'haplotypes ne permet de complètement différencier les uns des autres les stocks des rivières, ou les complexes régionaux, nous avons évalué la fréquence des haplotypes comme descripteur de stock. À l'analyse de quatre indices de divergence haplotypique uniques en leur genre, nous avons constaté qu'on obtient une meilleure résolution en incluant l'hétéroplasmie des sites de restriction au lieu de la supprimer; toutefois, cet élément n'a pas influé de façon marquée sur les résultats finaux. Par ailleurs, en raison de la répétition de la variation d'une enzyme de restriction à l'autre, la richesse d'information a vite diminué une fois passées les six meilleures enzymes, la cause de cette baisse étant la liaison physique des sites de restriction dans la molécule d'ADN mitochondrial. Nous avons vérifié le pouvoir de discrimination en calculant le rendement de l'affectation des divers caractères de l'ADN mitochondrial. Chaque sujet a été provisoirement exclu de l'ensemble de données et réaffecté aux diverses populations candidates en fonction de la similitude haplotypique : 28% des réaffectations se sont révélées correctes, ce qui est quatre fois plus que ce qu'on obtient avec l'affectation aléatoire. Nous avons démontré

Received May 24, 1994. Accepted March 3, 1995.
J12384

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que, même si l'identité spécifique du stock d'origine des individus ne peut être établie avec un degré de confiance suffisant, les variantes haplotypiques observées par rapport aux stocks de base permettent d'identifier les stocks et d'analyser les stocks mixtes dans le cas de l'alose, car les fréquences haplotypiques sont spécifiques aux stocks de chaque rivière.
[Traduit par la Rédaction]

Introduction

Accurate composition estimation within population mixtures is a desirable component of fishery management. Population mixtures occur either naturally (e.g., where reproductively isolated river populations collectively school in the open ocean) or artificially (e.g., where hatchery-reared individuals are translocated to formerly isolated recipient populations). Traditionally, mark-recapture methods with physical tags have been used to extrapolate mixture proportions (Fraidenburg and Lincoln 1985; Johnson 1990), while recent attention has focused on intrinsic biological markers (Smouse et al. 1990). Intrinsic markers are useful for stock discrimination if they are distributed in one of two ways: (*i*) they are differentially fixed for discernible character states, or (*ii*) they exhibit stable and substantial frequency differences among populations. Biochemical and molecular genetic markers routinely meet one or both of these criteria and avoid many of the weaknesses associated with environmentally influenced biological markers (Utter and Ryman 1993).

The foundation for mixed-stock analysis (MSA) in fisheries management is the stock concept (Berst and Simon 1981; Utter et al. 1973 and references therein). The stock concept provides that species are regularly composed of multiple subunits (hereafter, stocks) and that a stock exhibits spatial, temporal, or behavioral integrity from other such stocks (Berst and Simon 1981 and references therein). This integrity effectively permits (or may be caused by) divergence of gene pools. Because MSA profits from and is limited by the level of differentiation of individual genetic characters among stocks, improved resolution is often achieved by using several independently segregating markers. Yet, even with an extensive battery of markers such as a suite of allozymes, confident assignment of an individual to its correct stock of origin is often not possible. However, several multiple-locus investigations on Pacific salmon, *Oncorhynchus* spp. (Fournier et al. 1984; Wood et al. 1987; Shaklee et al. 1990; Brodziak et al. 1992), and lake trout, *Salvelinus namaycush* (Marsden et al. 1989), have documented the effectiveness of MSA to partition population mixtures into contributing stock proportions.

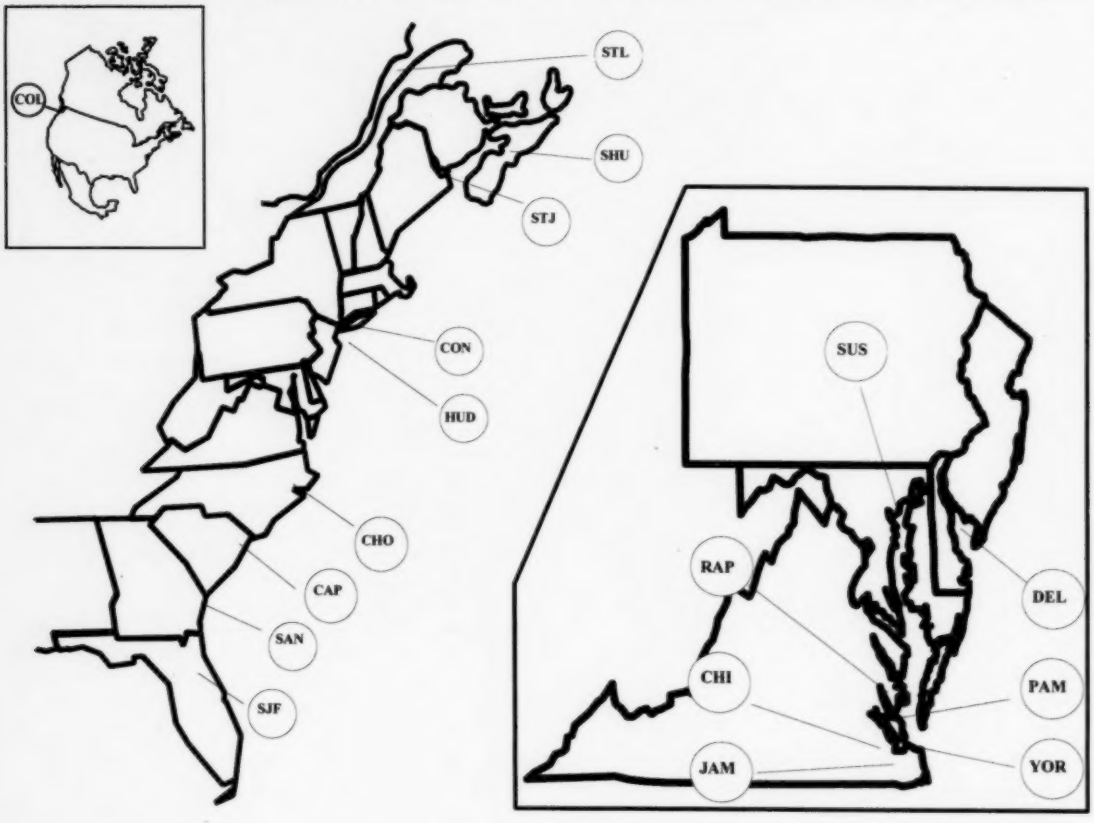
Attempts to develop genetic stock identification capabilities for anadromous Atlantic coast species, however, have met with only limited success. For example, distributions of allozyme and morphological characters indicated that Atlantic salmon, *Salmo salar*, cluster along broad geographic scales (e.g., European versus North American; Ryman 1983; Gyllensten 1985; Stahl 1987). For striped bass, *Morone saxatilis*, the observed distribution of morphological characters suggests a divergence among northern, middle, and southern Atlantic regional populations (Mansuetti and Kolb 1953; Setzier et al. 1980). On a smaller geographic scale, extensive allozyme analyses failed to resolve gene pool differences among putative east coast *M. saxatilis* stocks (Morgan et al. 1973; Grove et al. 1976; Sidell et al. 1980). In American shad, *Alosa sapidissima*, the distributions of morphological and life history characters indicated a north to south clinal gradient among river populations (Leggett and Carscadden 1978), while allozyme surveys revealed low levels of geographic variation and a clinal gradient for a single esterase-encoding locus (Shoubridge 1978). For all three species, allozyme variation is insufficiently divergent to support MSA of river-specific stocks.

The power of mitochondrial DNA (mtDNA) markers for discriminating among fish stocks has previously been demonstrated (Bentzen et al. 1989; Davidson et al. 1989; Schweigert and Withler 1990; Wirgin et al. 1990). mtDNA markers are potentially more sensitive than allozymes for population studies because mtDNA mutates and segregates among reproductive isolates more rapidly than does nuclear DNA (Brown et al. 1979). Furthermore, the rates of gene flow as a result of straying among stocks should be lower for mtDNA genes than for nuclear genes. Because mitochondria are maternally inherited, the differences between stocks are more readily observed (Birky et al. 1983, 1989; Bentzen et al. 1989). Moreover, most allozyme-encoding nuclear loci used for stock identification and MSA purposes exhibit only a few alleles, whereas most mtDNA investigations benefit from a large number of different mtDNA haplotypes, which potentially improves resolution.

Stock discrimination with a mtDNA data array requires two considerations. First is the absence of a well-established comparative reference frame, which strengthens mixed-stock analyses that use diploid markers. Haploid characters have been used extensively for comparing human admixtures to source populations (Roberts and Hiorns 1962, 1965; Long and Smouse 1983; Chakraborty and Smouse 1988; Long 1991; Chakraborty 1992), but these studies sacrifice statistical power because haploid characters cannot exhibit a Hardy–Weinberg reference frame or a Wahlund (1928) effect. The second consideration for use of mtDNA data is that all the polymorphic sites on the mtDNA molecule are physically linked and thus inherited as a non-recombinant haplotype. Each polymorphic site is thus statistically associated with each other polymorphic site. The lack of statistical independence translates into an informational redundancy that, in turn, translates into a diminishing informational return from the increased laboratory effort required to generate a multisite haplotypic array.

Unfortunately, allozyme surveys of American shad from across the species' native range revealed only minor genetic divergences either within or among river populations (Shoubridge 1978; Sismour and Birdsong 1986). Therefore, a robust diploid data set was not immediately available. Curiously, physical data, including reproductive characteristics and morphological traits, vary latitudinally across

Fig. 1. Sampling locations for American shad (*Alosa sapidissima*): STL, Saint Lawrence River, Quebec; SHU, Shubenacadie River, Nova Scotia; STJ, Saint John River, New Brunswick; CON, Connecticut River, Connecticut; HUD, Hudson River, New York; DEL, Delaware River, New Jersey; SUS, Susquehanna River, Maryland; RAP, Rappahannock River, Virginia; YOR, York River, Virginia; PAM, Pamunkey River, Virginia; CHI, Chickahominy River, Virginia; JAM, James River, Virginia; CHO, Chowan River, North Carolina; CAP, Cape Fear River, North Carolina; SAN, Santee River, South Carolina; SJF, Saint John's River, Florida; and COL, Columbia River, Washington.



shad's native range indicating an adaptive radiation and stock segregation (Carscadden and Leggett 1975; Leggett and Carscadden 1978; Melvin et al. 1986). Furthermore, Melvin et al. (1986) documented a high degree of reproductive philopatry (greater than 90%) suggesting a mechanism for geographic isolation.

Recent surveys of mtDNA variation, however, have revealed some evidence of population architecture in shad. Bentzen et al. (1988, 1989) examined the mtDNAs of 243 shad from 14 east coast river populations and demonstrated several single-enzyme mtDNA haplotypic differences within the present geographic range. Nolan et al. (1991) employed a different set of restriction enzymes on a geographically dispersed subset of those same river systems and obtained similar results. These investigators also documented that 58% of the individuals possessed unique mtDNA haplotypes. Brown and Chapman (1991) surveyed 549 shad from 10 mid-Atlantic rivers with a larger suite (15) of restriction endonucleases and observed significant divergence among most river populations, while demonstrating a large range of haplotypes (4–48%) that were specific to river sources.

Brown and Chapman (1991) also investigated the composition of shad harvested as part of Virginia's coastal intercept fisheries. A maximum likelihood analysis (GIRLSEM; J. Pella, National Marine Fisheries Service, personal communication) of population admixtures sampled from commercial harvests attributed 81% of the total 1991 catch to the 10 mid-Atlantic river populations. Conversely, 19% of the mixed-source harvest had unique mtDNAs not attributable to any of the sampled source populations. The authors concluded that either the baseline rivers were insufficiently sampled or the harvest contained shad from unsampled river sources.

Our objectives for the present study were to (*i*) investigate the level and structure of mtDNA restriction site variation in large samples of American shad and (*ii*) determine the most effective use of mtDNA restriction site variation for MSA by examining four different metrics of genetic divergence. By including a large number of

restriction enzymes in an initial survey battery, we further (*iii*) assessed how much informational redundancy was present within the assay battery and (*iv*) determined which subset of restriction endonucleases was most powerful for continued stock and harvest monitoring of American shad.

Methods

Field sampling

Field personnel from cooperating state, provincial, and federal agencies collected over 1000 individuals from 14 Atlantic coastal drainages and one west coast drainage in 1992 (Fig. 1). Sexually mature adults committed to upriver migration were targeted for collection to maximize the chance that collections represented discrete breeding populations. Samples (n = 23–117) were collected from rivers that traditionally supported large runs. Cooperators forwarded tissue samples to the laboratory in one of three forms: whole-fresh (on wet ice), dissected-fresh (on wet ice), or dissected-frozen (on dry ice). Columbia River, Washington, was included because this source was used in part to rehabilitate the Susquehanna River run. Columbia River shad were originally introduced from Sacramento River, California, in the 1890s and the Sacramento River population was itself established by an introduction of Hudson River shad in 1870 (Scott and Scott 1988). The invasion front of shad currently extends to northern Siberia.

Laboratory protocols

We isolated mtDNA from tissue samples with the total genomic DNA isolation protocol of Doyle and Doyle (1987). When eggs were assayed, we digested mtDNA samples with each of 14 restriction endonucleases (hereafter, REs; (*Aat*I = *Stu*I (5′-AGGCCT-3′), *Apa*I (GGGCCC), *Bcl*I (TGATCA), *Bgl*I (GCC (N)$_x$GGC), *Dra*I (TTTAAA), *Eco*RI (GAATTC), *Eco*RV (GATATC), *Hind*III (AAGCTT), *Kpn*I (GGTACC), *Pst*I (CTGCAG), *Pvu*II (CAGCTG), *Sal*I (GTCGAC), *Sma*I (CCCGGG), *Sst*II = *Sac*II (CCGCGG))), according to the manufacturers' instructions (Bethesda Research Laboratories, United States Biochemical, and New England BioLabs). We electrophoresed the digested mtDNA in 0.8–1.0% agarose horizontal gels overnight, stained with ethidium bromide, and visualized fragment patterns under ultraviolet light. For other tissues, DNA was transferred from each gel onto a nylon filter (Southern 1975), hybridized with a nonradioactive probe, and visualized with a colorimetric reaction (Blugene detection kit, Bethesda Research Laboratories).

Data analysis

We estimated individual mtDNA fragment sizes by comparison with molecular weight standards and recorded a fragment profile for each individual over all restriction enzymes. An individual's fragment profiles from the 14 restriction enzymes were combined to construct a composite haplotype. For individuals missing one of the 14 restriction endonuclease genotypes, we conservatively assigned an A genotype (i.e., the most common) and verified in each case that this approach did not create a novel haplotype.

Individual restriction-site maps and a composite map (see Bentzen et al. 1989 for the original) were constructed with double digests and inferred presence (1) or absence (0) of single restriction sites for each haplotype. From the presence and absence information, we computed the frequency of composite haplotypes and a haplotypic diversity measure from the equation

$$[1] \qquad H = 1 - \sum_i P_i^2$$

where P_i is the frequency of the ith haplotype.

We encountered length variation and length heteroplasmy in our samples, phenomena first described in shad by Bentzen et al. (1988). Heteroplasmy results from individuals possessing multiple mtDNA haplotypes. For our analyses, we suppressed both length variation and length heteroplasmy because these lack clearly established and validated rules of interpretation (Moritz et al. 1987), the net effect of which was to ignore a potential source of variation. Furthermore, the generational stability, mode of inheritance, and lability of length variation have not been thoroughly investigated for shad and inclusion in the analysis potentially confuses the issue of stock divergence. We included restriction site heteroplasmy in our analyses because mtDNA transmission models are available that account for restriction site heteroplasmy, originating either from paternal leakage of recent mutations within a single female generation (Takahata and Maruyama 1981; Chapman et al. 1982; Birky et al. 1983) or from a combination of selection and mutation (Clark 1988). Furthermore, a growing body of evidence has documented inheritance and temporal stability of the phenomenon in natural populations (Bermingham et al. 1986; Bentzen et al. 1988; Brown and Paynter 1991; Zouros et al. 1992).

We used an analysis of molecular variance, AMOVA (Excoffier et al. 1992), to gauge the overall diversity among populations and to partition haplotypic variation into components representing divergence among regional sets of rivers, divergence among rivers within a region, and variation within rivers. To investigate the effects of heteroplasmy on the analysis, we constructed and compared four different distance metrics (measures of divergence among haplotypes). Choice of an appropriate distance metric was driven by two considerations.

The first consideration was how best to treat heteroplasmic site variation. Heteroplasmy may be treated as a codominant character or as a dominant character. If heteroplasmy results from two co-occurring mitochondrial haplotypes within an individual, then scoring recognition sites as 2 for homoplasmic presence, as 0 for homoplasmic absence, or as 1 for heteroplasmic (or, effectively, codominantly heterozygous) sites would be appropriate. Alternatively, if heteroplasmy results from assay limitations (i.e., variation in the fidelity of restriction site recognition), reducing all data to a presence (1) or absence (0), or dominant form of coding is more appropriate.

The second consideration was whether the degree of mutational divergence among haplotypes conveys any useful information about genetic divergence among populations. Excoffier et al. (1992) documented that site-difference metrics (i.e., weighted by degree of divergence) resolved human population differences better than equidistant metrics (i.e., equally weighted). Recent theoretical results

(Slatkin 1995; Goldstein et al. 1995) suggest that if the exchange rate between populations is small, relative to the mutation rate, then site-difference metrics should perform better because geographically separated populations will have different constellations of alleles rather than different frequencies of alleles. Alternately, if the exchange rate is high, then the equidistant metrics should outperform the site-difference metrics because all alleles will be distributed widely and observed only as frequency differences among populations. We therefore constructed and compared four metrics: CED (for codominant equidistant), CSD (for codominant site-difference), DED (for dominant equidistant), and DSD (for dominant site-difference). The C metrics incorporate the restriction site heteroplasmy, whereas the D metrics ignore it.

The substrate for AMOVA was a 988 × 988 matrix whose elements were the distance coefficients between all pairs of individuals. Each of the four distance metrics yielded a characteristic matrix. To define the distance between the jth and lth individuals, we first defined a pair of genetic score vectors

$$[2a] \quad Y_j = [y_{j1}, y_{j2}, \ldots, y_{jK}]^T$$

and

$$[2b] \quad Y_l = [y_{l1}, y_{l2}, \ldots, y_{lK}]^T$$

of some length K, representing the genotypes of the jth and lth individuals, where the superscript T indicates the transpose of the vector. The squared distance between the jth and lth individuals is the squared distance between the two vectors, defined as

$$[3] \quad \delta^2_{jl} = [Y_j - Y_l]\cdot[Y_j - Y_l]^T$$

Each distance metric determined the length and suite of element values for the vector. For example, all $K = 116$ haplotypes are assumed to be equally divergent for the CED metric. An individual with the kth haplotype ($k = 1, \ldots, K - 1 = 115$) has a 1 in the kth position of the vector and 0's elsewhere; the final haplotype has a 0 in all 115 positions. For the DED metric, all of the 2's become 1's, and there are now only $K' = 89$ distinguishable haplotypes. The kth haplotype ($k = 1, \ldots, K' - 1 = 88$) has a 1 in the kth position and 0's elsewhere; the 89th haplotype has all 0's. For the CSD and DSD metrics, which account for the number of restriction site differences among haplotypes, the length of the vector is $K'' = 62$, which is the number of polymorphic restriction sites detected. In the case of CSD, the kth site has a 2 if homoplasmic and present, 1 if heteroplasmic, and 0 if homoplasmic and absent. For the DSD metric, both homoplasmic presence and heteroplasmy are represented by 1's.

Because we did not know, a priori, whether genetic variation in American shad was geographically organized, we explored several biologically plausible hypotheses for regional lumping. The first hypothesis separated primarily semelparous populations (drainages south of Chesapeake Bay) from iteroparous populations (Chesapeake Bay and northward). This scheme exhibited a minor genetic distinction, but the results were not statistically compelling. Several other biogeographical groupings that might be useful for interjurisdictional management purposes also failed to produce significant among-region components of variance.

To allocate an individual to a particular population, we computed the genetic distance between that individual and the averages of each of the populations. For the jth individual in the ith population, we computed the distance to the hth population with the following pair of genetic score vectors:

$$[4a] \quad Y_{ij} = [y_{ij1}, y_{ij2}, \ldots, y_{ijK}]^T$$

and

$$[4b] \quad \bar{Y}_{h\cdot} = [\bar{y}_{h\cdot 1}, \bar{y}_{h\cdot 2}, \ldots, \bar{y}_{h\cdot K}]^T$$

The mean vector $\bar{Y}_{h\cdot}$ is the vector of average scores for the hth population.

We allocated individuals to the genetically closest source population by computing the distance from the jth individual in the ith population to the mean of each population in turn. For the hth population ($h \neq 1$), we had

$$[5a] \quad \delta^2_{ij(h)} = [Y_{ij} - \bar{Y}_{h\cdot}]^T \mathbf{W}[Y_{ij} - \bar{Y}_{h\cdot}]$$

where $\mathbf{W}$ was the weight matrix described below. To compare an individual with its own population mean ($h = i$), we modified eq. 5*a* (Lachenbruch 1967):

$$[5b] \quad \delta^2_{ij(i)} = \left[\frac{N_i}{N_i - 1}\right]^2 [Y_{ij} - \bar{Y}_{i\cdot}]^T \mathbf{W}[Y_{ij} - \bar{Y}_{i\cdot}]$$

This modification removed the individual from the population and compared it with ($N_i - 1$) other individuals in the population sample. Equation 5*b* eliminates upward bias owing to the inclusion of the tested individual as part of the average for its own population. This bias can be substantial for small to moderate sample sizes, especially where there are large numbers of unique haplotypes. $\mathbf{W}$ was not adjusted for each individual because the correction is inconsequential with larger total sample sizes (Lachenbruch 1975).

The weighted matrix $\mathbf{W}$ for both equations was the inverse of the within-population covariance matrix $\mathbf{V}$ for the specific metric in question:

$$[6] \quad \mathbf{V} = \sum_{i=1}^{15} \sum_{j=1}^{N_i} [Y_{ij} - \bar{Y}_{i\cdot}]\cdot[Y_{ij} - \bar{Y}_{i\cdot}]^T/(N - 15)$$

For the CED metric, $\mathbf{W}$ and $\mathbf{V}$ are of dimension $K - 1 = 115$; for DED, $\mathbf{W}$ and $\mathbf{V}$ are of dimension $K' - 1 = 88$; and for CSD and DSD, $\mathbf{W}$ and $\mathbf{V}$ are both of dimension $K'' = 62$.

Using eqs. 5*a* and 5*b*, we computed the distance between each individual and the candidate populations and then allocated the individual to the population that was least distant. Ties were apportioned equally to the corresponding populations. Finally, the fractions of correctly allocated individuals were tallied as a measure of success. A high success rate indicated strong genetic stock divergence and considerable statistical power and utility for subsequent mixed-stock analysis, whereas a low success rate implied minimal genetic stock divergence and low statistical power for mixed-stock analysis.

We performed an additional a posteriori test for sampling sufficiency with a combinatorial approach similar to that of Hebert et al. (1988) and Bernatchez et al. (1989).

The relationship between haplotype diversity and sample size was assessed by randomly selecting subsamples of 10, 20, 30, etc., individuals (without replacement) from each population and counting the number of haplotypes uncovered. We repeated this process 1000 times for each population to obtain the mean number and standard deviation of haplotypes detected at each level of sampling intensity.

Results and discussion

Composite haplotype variation

We obtained complete or nearly complete 14-enzyme assay batteries on each of 988 individuals. Restriction site analysis produced 116 haplotypes from the 15 drainages (Table 1). The most common haplotype (designated AAAAAAAAAAAAAA) occurred at an overall frequency of 51.3%, but the values for individual sources ranged from 15.0 to 73.8%. Seven other haplotypes occurred at frequencies between 1.0 and 10.9%; the remainder were rare (<1.0%) and occurred only once or a few times in single populations. Each population exhibited a different array for haplotypic frequency and a characteristic assemblage of rarer haplotypes.

In total, we detected 100 RE sites from the 14 REs, 62 of which were polymorphic in these populations (Table 2). Some enzyme character sets (*Apa*I, *Eco*RI, *Eco*RV, *Kpn*I, and *Sal*I) were more variable ($H > 0.07$) than others and thus were better candidates for detecting differences among populations.

Partitioning of molecular variance

Three conclusions emerged from the AMOVA results for all four metrics and the complete battery of REs (Table 3). (*i*) Although the vast majority of variation occurred within populations (94–96%), genetic differences among populations were highly significant ($\alpha \leq 0.001$ in all cases). (*ii*) The codominant (2,1,0) metric allocations performed slightly better than the dominant (1,0) metrics. (*iii*) The fraction of variation separating populations was greater with site-difference metrics (CSD and DSD) than with equidistant metrics (CED and DED), but the improvements were small. Overall, incorporating either the heteroplasmic information or the degree of haplotypic divergence into the analysis had little impact on resolving power and the results did not depend in any important way on the precise choice of metric.

Genetic divergence among populations

The 15-population AMOVA provided an overview of the divergence among populations, but did not convey a detailed pattern of interstock differences. AMOVA indicated that the level of interstock differentiation (a highly significant ϕ_{ST}) ranged from 3.95 to 5.62%, depending on the distance metric used, suggesting a meaningful, albeit subtle, stock structure for shad. By computing AMOVAs for particular pairs of populations, however, we obtained estimates of genetic distance between each population pair. The upper triangular portion of Table 4 presents the DED distances (square roots of the between-population variance components) among all population pairs; the lower triangular portion presents the corresponding degree of statistical significance. Several pairwise distances yielded negative variances, which indicate that they were not significantly different from zero (at the $\alpha < 0.05$ level; these are indicated by three asterisks). We expected some relationship between genetic distance and geographic proximity (i.e., lower distance values between geographically proximal rivers), but the distances appear to be randomly distributed relative to geography. This absence of a clear and orderly biogeographic pattern probably resulted from two causes: (*i*) relatively free, albeit limited, exchange between river sources since the last period of glaciation (see Melvin et al. 1986), and (*ii*) shad management practices over the last 100 years, which may have partially homogenized once-meaningful geographic differences.

Selecting a set of restriction enzymes

Ideally, we would prefer the capacity to allocate individual fish from a mixed harvest to their correct sources on the basis of mitochondrial haplotype. The raw data (Table 1) show that a completely accurate allocation of isolated individual fish is not feasible, even with an extensive battery of markers. In fact, adding to the enzyme battery did not greatly increase resolving power and may have obscured differences if the addition created new, but rare, haplotypes. Regardless, Smouse et al. (1982) documented that allocation success increases predictably with increasing average genetic distance between pairs of populations. It has also been demonstrated that the power of MSA increases with the average genetic distance between populations (Smouse et al. 1990). As a result, correct allocation fractions serve as convenient measures of the genetic resolution and translate into statistical power for MSA.

Smouse et al. (1982) documented the linearity between the probit of the correct allocation fraction (denoted π here) and average genetic distance (D) between pairs of populations. For any two populations, random allocation success is $\pi = 1/2 = 0.50$, even in the absence of genetic data. With a larger number of candidate populations (say J), random allocation success is expected to increase (i.e., $\pi = 1/J$). Since $J = 15$ here, the random correct allocation fraction of $\pi = 1/15 = 0.067$ serves as the null hypothesis (no genetic divergence) reference value; any value of $\pi > 0.067$ indicates some genetic divergence. A larger value for π indicates a greater genetic divergence.

Smouse et al. (1982) demonstrated that the best approach for increasing population resolution for a given average level of genetic divergence per polymorphic marker is to increase the number of independent polymorphic markers. Lynch and Crease (1990) arrived at much the same conclusion for (nonindependent) restriction sites in *Daphnia pulex* mtDNA. As a first approximation, we might expect that adding ever more polymorphic REs should improve allocation success, genetic resolution, and the efficacy of MSA. Theoretically, if enough characters were added, genetically divergent populations could be characterized as nonoverlapping collections of unique haplotypes. Beyond a certain point, however, the sampling variation (i.e., system noise) inherent in characterizing large numbers of characters on small samples of individuals overwhelms the variation resulting from stock divergence (i.e., signal).

Table 1. Haplotype counts by population site.

	Haplotype	STL	SHU	STJ	CON	HUD	DEL	SUS	RAP	YOR	JAM	CHO	CAP	SAN	SJF	COL
1	AAAAAAAAAAAAA	69	28	42	33	19	48	64	9	43	23	3	16	29	22	59
2	AAAAAAAAAAAAB			1							1					
3	AAAAAAAAAAABA			4												
4	AAAAAAAAAAACA													1	1	
5	AAAAAAAAAAA/DAA			1												
6	AAAAAAAAAABAA	2	1	11	1		2	1	1	1	2		2			3
7	AAAAAAAAAADAA					1										1
8	AAAAAAAAAA/EAAA			1										2		
9	AAAAAAAAABAAA				2	1		3		1	1			2		
10	AAAAAAAAAEAAA	1														
11	AAAAAAAAAA/BAAAA														2	
12	AAAAAAAAABAAAA							1							2	
13	AAAAAAAAABADAA							1								
14	AAAAAAAAABA/EAAA													1		
15	AAAAAAAAADAAAA	4														
16	AAAAAAAAAHAA/DAA				1											
17	AAAAAAAAA/BAAAAA	1			1	1							2	1		
18	AAAAAAAAA/BAAACA												1			
19	AAAAAAAAA/BAAA/BAA										1					
20	AAAAAAAAA/BAABAA													1		
21	AAAAAAAABAAAAA	37	7	10	8	1	13	2	2	11	5	1	3	3	4	1
22	AAAAAAAABAAA/DAA										1					
23	AAAAAAAABAABAA		5	6	14	2	13	2	3	8	4	1	4	6	1	5
24	AAAAAAAABAABAB						1	1					1			
25	AAAAAAAABADBAA				2											
26	AAAAAAAABBAAAA									1						
27	AAAAAAAADAAAAA									1				1		
28	AAAAAAAADAABAA									1						
29	AAAAAAAAEAAAAA	1								1						
30	AAAAAAAAEAABBA			1												
31	AAAAAAA/BAAAAAAA		2										2			3
32	AAAAAAA/BAAAADAA						1									
33	AAAAAAA/BAA/BAAAAA												2			
34	AAAAAAA/BABAAACA												1			
35	AAAAAAA/BABAABAA				1											
36	AAAAAABAAAAAAA		2	17	14	1	4	4	1	3	2		2	1		1
37	AAAAAABAAAABAA		1													
38	AAAAAABAABAAAA														2	
39	AAAAAABAA/BAAAAA			1	1											
40	AAAAAABABAAAAA				1											
41	AAAAAABABAABAA					2	1									
42	AAAAAABAEAAAAA			1												
43	AAAAAABCAAADAA															1
44	AAAAAACAAAAAAA							1								
45	AAAAAADAAAAAAA														2	
46	AAAAAADAAA/BAAAA							1								
47	AAAAAA/BAAAAAAA							1							1	
48	AAAAAA/BAABAABAA													1		
49	AAAAAA/BA/BAAAAACA												1			
50	AAAAABAAAAAAAA				1		2	2	1						7	
51	AAAAABAAAAAACA							1								
52	AAAAABAAABAAAA														1	
53	AAAAACAAAAAAAA						1									
54	AAAAADAABAAAAA					1										
55	AAAAAEAAAABAAA					1										
56	AAAAAEAABAABAA					6										
57	AAAAAGAAAAAAAA					1										
58	AAAACAAAAAAAAA							1								
59	AAAACAA/BAAAAAAA			1												
60	AAAA/BAAAABAABAA						1									
61	AAAA/EAAAAAAAAAA								1	1		4				
62	AAAA/EAABAAAAAAA											1				

Table 1 (*concluded*).

	Haplotype	STL	SHU	STJ	CON	HUD	DEL	SUS	RAP	YOR	JAM	CHO	CAP	SAN	SJF	COL
63	AAABAAAAAAAAAA						1									
64	AAAEAAAAAAAAAA	1										2				
65	AAAEAAAABAAAAA											2				
66	AAAEAAAABAABAA											2				
67	AAAFAAAAAAAAAA									1						
68	AAAFAAAABAABAA								1							
69	AAAFAABAAAAAAA			1												
70	AAAIAAAABAAAAA	1														
71	AAAJAAAABAAAAA									1						
72	AAA/BAAAAAAAAAAA													1		
73	AAA/BAAAAABAABAA													1		
74	AABAAAAAAAAAAA					1										
75	AABAAAAABAAAAA						1									
76	AABAAABAAAAAAA				1						1					
77	AACAAEAAAABAAA					1										
78	AADAAAAAAAABAA										1					
79	AAFAAAAAAAAAAA								1							
80	AAFAAAAAAAABAA				1											
81	AAGAAAAAAAABAA											1				
82	AA/BAAAAAAAAAAA							2								
83	AA/BAAAAAAAAAA/DAA										1					
84	AA/BAAAAAABAAAAA								1							
85	ABAAAAAAAAAAAA							3		1				1	8	
86	ABAAAAAAAAADAA															1
87	ABAAAAAAABAAAA									1	1				1	
88	ABAAAAAAABACAA								1							
89	ABAAAAAABAAAAA														2	
90	ABAAAAAABAAA/BAA										1					
91	ABAAAAAABAABAA														1	
92	ABAAAAAABAADAA		1													
93	ABAAAAAABA/BAAAA										1					
94	ABAAAABAAAAAAA										1					
95	ABAAAABAABAAAA							1								
96	ABAAABAAAAAAAA														1	
97	ABAABBAAAA/BAA/DAA						1									
98	ACAAAAAAAAAAAA														4	
99	ACAAAAAAAA/BAAAA														1	
100	ACAAAAAABAAAAA				1										1	
101	ACAAABAAAAAAAA														2	
102	ADAAAAAAAAAAAA							2								
103	AFAAAABAAAAAAA							1								
104	BAAAAAAAAAAAAA								1							
105	BAAAAAAAA/BAAAAA														1	
106	BAAFABAAAAAAAA														1	
107	DAAAAAAAAAAAAA						3	3		1	2	1				4
108	DAAAAAAAAAABAA					1										
109	DAAAAAABAAAAAA						1									
110	DAAAABAAAAAAAA															1
111	DAAA/EAAAAAAABAA											1				
112	DAAEAAAABAAAAA											1				
113	DBAAAAAAAAAAAA														1	
114	DBAAAAABBAAAAB									1						
115	EAAAAAAAAAADAA		1													
116	GAAAAAAABAABAA						1			1						
Total counts (*N*)		117	48	98	83	40	95	98	23	79	49	20	37	52	69	80

Note: In statistical analyses, we pooled YOR with PAM and JAM with CHI, because they are within the same drainage basin. Haplotypes are composites of individual REs listed in alphabetic order: *Aat*I; *Apa*I; *Bcl*I; *Bgl*I; *Dra*I; *Eco*RI; *Eco*RV; *Hin*dIII; *Kpn*I; *Pst*I, *Pvu*II, *Sal*I, *Sma*I; *Sst*II.

Table 2. Counts (N) of single-enzyme haplotypes (including site presence and absence designation) recovered from source populations of American shad (*Alosa sapidissima*), and associated heterozygosity values (H), computed from eq. 1 of the text.

Restriction enzyme	Haplotype designation	Number	H
*Aat*I	A 101101111	961	0.053
	D 101101011	21	
	B 001000001	3	
	G 101001111	2	
	E 001011111	1	
*Apa*I	A 1100100	946	0.082
	B 1100000	30	
	C 1000000	9	
	D 1101100	2	
	F 1111110	1	
*Bcl*I	A 10010101	979	0.018
	B 10010001	4	
	F 10010111	2	
	C 10101101	1	
	D 11010101	1	
	G 00010100	1	
*Bgl*I	A 11010001110000	972	0.032
	E 11010000110000	8	
	F 11110001110000	4	
	B 11011001110000	2	
	I 11010011110000	1	
	J 01011001101111	1	
*Dra*I	A 100110111	985	0.006
	C 100111111	2	
	B 100010111	1	
*Eco*RI	A 10000110	952	0.071
	B 10010110	25	
	E 10000010	8	
	C 10000111	1	
	D 10110110	1	
	G 10000100	1	
*Eco*RV	A 10010	900	0.163
	B 10011	84	
	D 10110	3	
	C 11010	1	
*Hin*dIII	A 10000	985	0.006
	B 10111	2	
	C 11111	1	
*Kpn*I	A 11101000	755	0.364
	B 10001000	226	
	E 10101000	4	
	D 11001000	3	
*Pst*I	A 101111	969	0.038
	B 100111	15	
	D 101011	4	
*Pvu*II	A 110001	969	0.038
	B 110000	12	
	E 111001	5	
	D 110111	2	
*Sal*I	A 1011	845	0.250
	B 1010	134	
	D 0011	8	
	C 0110	1	
*Sma*I	A 100000	977	0.022
	C 100100	6	
	B 111111	5	
*Sst*II	A 10011	982	0.012
	B 01111	6	

Table 3. Mitochondrial analysis of molecular variance (AMOVA) for restriction site polymorphisms in American shad (*Alosa sapidissima*), based on four different genetic distance metrics: codominant equidistant (CED), codominant site-difference (CSD), dominant equidistant (DED), and dominant site-difference (DSD).

Metric	Source of variation	Sums of squared deviations	Mean squared deviations	Percentage variance components
CED	Populations	18.75	1.34	4.28
	Individuals	334.26	0.34	95.72
CSD	Populations	104.40	7.81	5.62
	Individuals	1563.58	1.61	94.38
DED	Populations	17.12	1.22	3.95
	Individuals	324.38	0.33	96.05
DSD	Populations	50.92	3.64	5.21
	Individuals	774.80	0.80	94.79

Note: Pamunkey River data were pooled with York River data; Chickahominy River data were pooled with James River data. Within each metric, there were 14 degrees of freedom associated with populations and 973 degrees of freedom associated with individuals. Each metric was significant at the $\alpha \leq 0.001$ level.

More directly, credible estimation requires many more individuals than characters! Additional resolution is lost because RE sites on the mtDNA do not segregate independently; as suggested earlier, large numbers of mtDNA restriction sites may be informationally redundant. The essential strategy is to choose a subset of REs that optimizes genetic divergence, relative to the within-population sampling variation. The critical question of how much RE variation is enough to differentiate among stocks required an allocation exercise with different subsets of REs (Table 2). Again, each individual was allocated to that source population (out of 15) for which the distance was smallest, using eqs. 5*a* and 5*b* for each of the distance metrics.

As we suggested earlier, the tallies indicated that *Apa*I, *Eco*RI, *Eco*RV, *Kpn*I, and *Sal*I were more likely to resolve stock differences than the others (Table 2). The only way to be absolutely sure that the optimal RE battery was identified was to explore all $(2^{14} - 1)$ combinations of the 14 RE sets, an intractable proposition. As a practical matter, we exhaustively explored all single REs, all two-RE sets, and all three-RE sets, but from that point onward, we explored further expansion of the battery in a stepwise manner (Table 5). We observed that while the correct allocation percentages and precise cascade of enzymes differed for the four distance metrics, a natural cascade emerged that was robust to the choice of metric (Table 5).

The best cascade of enzymes began with *Sal*I, followed by *Sal*I + *Apa*I, and then *Eco*RI + *Kpn*I + *Sal*I. Beyond this point, informational redundancies became important, and we found that adding the less-polymorphic REs, rather than additional highly polymorphic (but redundant) REs,

Table 4. Estimated genetic distances among all possible pairs of American shad (*Alosa sapidissima*) populations (designations are as in Fig. 1).

	STL	SHU	STJ	CON	HUD	DEL	SUS	RAP	YOR	JAM	CHO	CAP	SAN	SJF	COL
STL	—	0.17	0.28	0.31	0.28	0.21	0.29	0.22	0.18	0.20	0.33	0.25	0.23	0.32	0.32
SHU	0.04	—	0.13	0.13	0.14	***	0.14	***	***	***	0.20	0.03	***	0.24	0.17
STJ	<0.01	0.08	—	0.08	0.17	0.14	0.25	0.02	0.16	0.10	0.15	***	0.17	0.22	0.29
CON	<0.01	0.05	0.14	—	0.18	0.11	0.29	***	0.16	0.11	0.14	***	0.17	0.21	0.32
HUD	0.01	0.08	0.03	0.02	—	0.13	0.21	0.05	0.13	0.08	0.12	0.13	0.12	0.18	0.27
DEL	<0.01	0.63	0.02	0.04	0.08	—	0.19	***	***	***	0.16	***	***	0.21	0.23
SUS	<0.01	0.07	<0.01	<0.01	0.01	<0.01	—	0.22	0.14	0.17	0.29	0.25	0.10	0.29	0.02
RAP	0.05	0.50	0.41	0.55	0.33	0.91	0.02	—	***	***	***	***	***	0.10	0.29
YOR	0.02	0.96	0.01	0.03	0.09	0.76	0.03	0.60	—	***	0.19	0.08	***	0.22	0.19
JAM	0.03	0.60	0.16	0.11	0.23	0.77	0.02	0.91	0.81	—	0.10	***	***	0.17	0.23
CHO	0.01	0.06	0.11	0.13	0.16	0.09	<0.01	0.48	0.07	0.21	—	0.09	0.19	0.12	0.36
CAP	0.01	0.38	0.85	0.87	0.10	0.36	<0.01	0.81	0.21	0.56	0.27	—	0.12	0.18	0.30
SAN	0.01	0.64	0.01	0.02	0.13	0.44	0.10	0.49	0.84	0.58	0.04	0.11	—	0.22	0.15
SJF	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.16	<0.01	0.01	0.14	0.01	<0.01	—	0.36
COL	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	0.33	<0.01	0.01	<0.01	<0.01	<0.01	0.04	<0.01	—

Note: Genetic distances between pairs of populations (DED for 89 haplotypes) are listed above the diagonal, and pairwise random probabilities of larger values below the diagonal; <0.01 indicates a probability less than 0.01; *** indicates a negative among-population variance component estimate.

occasionally added the greatest resolution. A stepwise exploration indicated that the best quartet was *Bgl*I + *Eco*RI + *Kpn*I + *Sal*I, followed by the quintet *Bgl*I + *Eco*RI + *Kpn*I + *Pst*I + *Sal*I. Some modest gains were available for the equidistant metrics (CED and DED), which peaked at 28.4% (13 REs) and 23.9% (14 REs), respectively. By contrast, further gains were minimal with the site-difference metrics (CSD and DSD), which peaked at 23.1% (8 enzymes) and 21.7% (13 enzymes), and then declined to 21.0 and 20.2%, respectively, for the full 14-enzyme battery. Obviously, mtDNA markers do not permit assignment of an individual fish to a source with a high degree of confidence, but the use of genetic information clearly improves the success rate, relative to random allocation (6.67% for $J = 15$ sources).

Importantly, we achieved only small to modest improvements in allocation success (and thus genetic resolution) from adding more than four to five REs, because of the informational redundancy inherent in a set of rigidly linked and nonrecombining genetic markers. Pursuing additional variable mtDNA sites for American shad is not an economically promising strategy. The likelihood of uncovering source-diagnostic mtDNA markers is low, in view of the distributions we have already uncovered. Additional REs represent an incremental increase in expense, while adding minimal additional resolution. Even a strategy that focuses on four-base restriction enzymes has failed to reveal major geographic patterns not already observed with six-base cutters (Nolan et al. 1991).

Because individual shad were misallocated about 72–80% of the time (depending on the metric used), we sought to establish a pattern among the errors (Table 6). Some misallocations were to co-regional populations, particularly for Canadian river populations, but this same pattern was not applicable in southern U.S.A. Misallocated Chesapeake Bay region fish, for example, were not preferentially allocated to other Chesapeake Bay sources, but rather to the Columbia River. The single greatest determinant of allocation success (with any of the metrics and any combination of REs) was the placement of individuals with the AAAAAAAAAAAAA haplotype (most common, representing 51% of the total 1992 collection) into the population having the highest frequency of that haplotype (COL in this instance). Fish with other haplotypes were allocated to whichever populations had the highest relative frequencies of those haplotypes.

Sampling intensity

Brown and Chapman (1991) suggested that the presence of unique genotypes in Virginia's 1991 mixed-stock shad harvest resulted either from inadequate samples of the reference populations or from missing stocks in the baseline data set. Smouse et al. (1990) demonstrated how departures from Hardy–Weinberg equilibrium could be used to detect missing stocks in the baseline data set for diploid loci. Because a Hardy–Weinberg reference frame is not available for haploid mtDNA, we lack a suitable indicator that an unsampled baseline population is contained in a mixed harvest. The safest recourse is both to increase sample sizes of the stock collections and to attempt collection from all river populations. On the basis of their results with sample sizes of 30–50 individuals per spawning population, Brown and Chapman (1991) suggested enhancing sample sizes to at least 100 individuals per source population.

Larger sample sizes have several beneficial effects. First, larger sample sizes reduce the standard errors of frequency estimates for common haplotypes that provide most of the available power for MSA, and they also identify low-frequency haplotypes that are population or region specific. While singletons provide limited resolution, low-frequency stock-specific variants, called private polymorphisms

Table 5. Correct allocation percentages for different batteries of restriction enzymes, using four genetic distance metrics: codominant equidistant (CED), codominant site-difference (CSD), dominant equidistant (DED), and dominant site-difference (DSD).

Enzymes used		Codominant metrics			Dominant metrics		
		Haplotypes[a]	CED	CSD	Haplotypes[a]	DED	DSD
No genetic data							
		0	6.67	6.67	0	6.67	6.67
Single-enzyme batteries							
*Aat*I	(1)	5	8.15	8.15	5	8.15	8.15
*Apa*I	(2)	6	8.85	8.85	5	8.85	8.85
*Bcl*I	(3)	7	7.80	7.55	6	7.37	7.37
*Bgl*I	(4)	8	8.05	8.05	6	7.36	7.36
*Dra*I	(5)	3	5.89	5.89	3	5.89	5.89
*Eco*RI	(6)	7	9.26	9.36	6	9.36	9.36
*Eco*RV	(7)	5	12.75	12.15	4	12.15	12.15
*Hin*dIII	(8)	3	6.10	6.10	3	6.10	6.10
*Kpn*I	(9)	5	9.92	9.41	4	9.41	9.41
*Pst*I	(10)	4	6.76	6.76	3	7.06	7.06
*Pvu*II	(11)	5	6.00	5.98	4	5.98	5.98
*Sal*I	(12)	6	13.06	12.85	4	12.85	12.85
*Sma*I	(13)	3	7.23	7.23	3	7.23	7.23
*Sst*II	(14)	2	5.76	5.76	2	5.76	5.76
Multiple-enzyme batteries							
(2,12)		16	15.79	15.38	10	15.38	15.38
(9,12)		17	16.80	13.26	11	15.89	13.26
(6,9,12)		26	19.64	16.40	18	18.72	16.30
(4,6,9,12)		38	21.36	19.64	28	19.84	18.93
(4,6,9,10,12)		46	22.67	20.55	33	20.75	19.64
(3,4,6,9,10,12)		55	23.38	20.75	40	21.26	19.84
(1,3,4,6,9,10,12)		64	23.68	21.26	48	21.26	20.34
(1,3,4,6,7,9,10,12)		84	26.11	23.08	60	23.18	20.04
(1–12,14)		112	28.44	21.66	85	23.18	20.55
(1–14)		116	27.23	21.05	89	23.89	20.24

[a]Number of distinguishable haplotypes.

(Neel 1978), send clear signals. In fact, the distinction between a singleton and a private polymorphism is often one of sample size. Increasing the sample size also increases the recovery of a larger fraction of the rare haplotypes that exist within each stock, most of which will occur as additional singletons. Singletons will remain a sampling obstacle whether they are source-restricted variants, or whether they are rare and widely dispersed. Fortunately, Xu et al. (1994) documented for mtDNA that when stock sample sizes exceed 100, the further improvements to be gained in mixed-stock fishery analysis are minimal. In fact, when $n > 100$, frequencies of the common types stabilize. Additional rare haplotypes provide little additional stock resolution and do not enhance a MSA.

The test for sample size sufficiency indicated that our sample sizes exceeded the minimum for establishing precise mean frequencies. The sample sizes necessary to detect 95% of the haplotypes ranged from 30 to 90 and stabilized near 70 for most populations. As predicted, the number of rare variants increased with increasing sample size (Fig. 2). Notably, the relative frequencies of the most common haplotypes within a given population stabilized as sample sizes approached 40 (Fig. 3).

The implications of these results may at first run counter to our expectations. For a given sample size, the addition of restriction sites from a battery of new enzymes is expected to reveal novel haplotypes. However, the number of haplotypes uncovered (including rare singletons) will eventually overwhelm sample size within a population. Therefore, the collective frequencies of the rare haplotypes are more important than the frequencies of specific singletons. Clearly, the number of undiscovered haplotypes is potentially quite large and is an unknown function of sample size, as well as the number of polymorphic REs employed.

The appeal of rare haplotypes as a signature of MSA baseline populations may be erroneous. A statistical difficulty emerges from the allocation analyses we described

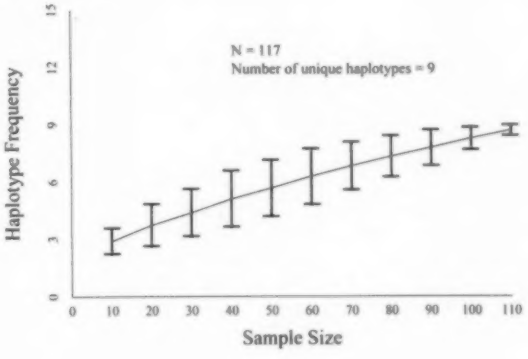
Table 6. Distribution of correct allocation fractions.

From source	To source														
	STL	SHU	STJ	CON	HUD	DEL	SUS	RAP	YOR	JAM	CHO	CAP	SAN	SJF	COL
STL	43		2						1		1	1			69
SHU	7	3	3	5								2			28
STJ	10		20	24						1			1		42
CON	8		16	20						1		1	2	2	33
HUD	1	1		2	14							1	1		20
DEL	13		6	13	1	7			1			1		2	51
SUS	2		5	2			11					1	3	7	67
RAP	2		2	3				5			1			1	9
YOR	2		5	3		1			4		1				21
JAM	15		4	10						10			3	1	48
CHO	1		1								14				4
CAP	3	2	4	4		1						7			16
SAN	3		1	6			2			1		1	7	2	29
SJF	4			2			1			1			1	38	22
COL	1		4	5	1							3			66

Note: An entry represents the number of individuals from a particular source population (row) that are allocated to each of the 15 populations (columns); PAM has been pooled with YOR, and CHI has been pooled with JAM. A discriminant analysis of the codominant equidistant metric (CED) and the full battery of 14 restriction enzymes were used. The diagonal is the number of individuals correctly allocated to the proper source.

earlier. The effect of removing a reference singleton from its own source population array, via eq. 5*b*, is that no useful information remains with which to reallocate it. The point of the removal is that the singleton's presence or absence in the sample conveys no useful information; it is largely a matter of stochastic chance. With increased sampling intensity, we can expect that some of these singletons will become private polymorphisms, and the allocation of a private polymorphism to its correct population is ensured. Unfortunately, more intense sampling frequently reveals additional singletons, confirming that finite sampling has not begun to exhaust the supply of rare variants. We demonstrated that frequencies of the 14-enzyme polymorphic mtDNA haplotypes stabilized at sample sizes of approximately 50–70. Even the cumulative frequency of singletons stabilized at these moderate sample sizes. Beyond a stock sample size of about $n > 100$, we would do better to direct additional field sampling to the elucidation of temporal and geographic information within the species.

Fig. 2. Relationship between sample size and mtDNA variation (mean frequency of haplotypes) detected for American shad (*Alosa sapidissima*) from Saint Lawrence River, Quebec. The bars indicate associated standard deviations.

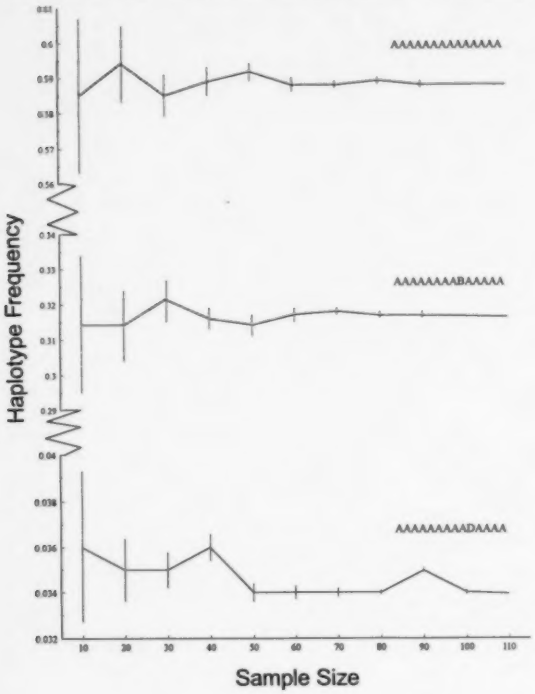


Prospects for mixed-stock fishery analysis

Utter and Ryman (1993) described sampling and analytical requirements for conducting a thorough mixed-stock analysis, including a clear and efficient ability to detect reliable information among populations. A complete categorical assignment of individuals is preferred, when possible, but it is not an absolute necessity. Thus, the level of divergence among populations is directly related to the level of resolution available for MSA. Most current methods (devised primarily for nuclear, biparentally inherited loci) employ some form of maximum likelihood procedure (Milner et al. 1985; Fournier et al. 1984; Millar 1987; Pella and Milner 1987; Wood et al. 1987; Smouse et al. 1990). The methods have been largely validated for chinook salmon (*Oncorhynchus tshawytscha*) by comparing results with coded wire tag data (Brodziak et al. 1992). Related methods based on a least-squares approach have recently been deployed for mtDNA haplotypes (Wirgin et al. 1993; Xu et al. 1994).

As with any other set of genetic markers, mtDNA markers will be effective for some species, but not for others, owing to the biological nature of the organism, and this is a matter for empirical examination. Therefore, the data should be tested before commencing a mixed-stock analysis. Our results suggest that there is sufficient genetic divergence among populations of American shad to provide about 28% correct allocation of individual fish. That level of

Fig. 3. Mean relative frequencies, in relation to sample size, for the three most common haplotypes of American shad (*Alosa sapidissima*) from Saint Lawrence River, Quebec. The bars indicate associated standard deviations.



genetic resolution is in keeping with the highly significant among-population components of the AMOVA (Table 3).

Our ultimate question was whether mixed-stock shad fisheries can be effectively delineated with mtDNA data. We anticipated that a genetic distance metric based on restriction site differences would increase resolution among populations, but such metrics did not vastly improve resolution for shad. There are many rare mtDNA variants in shad, but they show no discernible biogeographic clustering that makes sense in evolutionary (mutation, drift, isolation) terms. In retrospect, it seems clear that the level of genetic exchange among sources has been sufficient to preclude such structure, in keeping with the theoretical comments of Slatkin (1995) and Goldstein et al. (1995). The cause of this elevated exchange rate, resulting from either infidelity of spawning migration or deliberate stock transfers, remains unclear. Regardless of cause, the site-difference metric is not helpful in this species.

Regardless, we documented that shad gene pools in the various rivers overlap considerably, but there is nevertheless sufficient displacement among their means to support the MSA enterprise. We also documented (as a first approximation) that a subset of the polymorphic REs might be the most powerful for MSA. The informational redundancy among multiple polymorphic restriction sites indicates that little resolution was gained by adding REs. Finally, the limitations of mtDNA technology are inherent in the numbers, transmission, and distribution of mutations of the mitochondrial genome, rather than the number of restriction enzymes employed to detect variation.

Acknowledgments

We thank Arthur J. Butt, Jack Travelstead, Issac Wirgin, Peter Grewe, and an anonymous reviewer for helpful comments on earlier drafts of the manuscript. We also thank the cooperating agencies and personnel who collected shad samples and several students at Virginia Commonwealth University for laboratory assistance. The Virginia Marine Resources Commission (VMRC-F-110-R) and the Chesapeake Scientific Investigations Foundation (CSIF-VCU-92-010, CSIF-VCU-93-012) funded J.M.E. and B.L.B.; the New Jersey Agricultural Experiment Station (NJAES-32102/USDA) funded P.E.S. and C.J.K.

References

Bentzen, P., W.C. Leggett, and G.C. Brown. 1988. Length and restriction site heteroplasmy in the mitochondrial DNA of American shad (*Alosa sapidissima*). Genetics, 118: 509–518.

Bentzen, P., G.C. Brown, and W.C. Leggett. 1989. Mitochondrial DNA polymorphism, population structure, and life history variation in American shad (*Alosa sapidissima*). Can. J. Fish. Aquat. Sci. 46: 1446–1454.

Bermingham, E., T. Lamb, and J.C. Avise. 1986. Size polymorphism and heteroplasmy in the mitochondrial DNA of lower vertebrates. J. Hered. 77: 249–252.

Bernatchez, L., J.J. Dodson, and S. Boivin. 1989. Population bottlenecks: influence on mitochondrial DNA diversity and its effect in coregonine stock discrimination. J. Fish Biol. 35A: 233–244.

Berst, A.H., and R.C. Simon. (*Editors*). 1981. Proceedings of the Stock Concept Symposium. Can. J. Fish. Aquat. Sci. 38: 1457–1921.

Birky, C.W., T. Maruyama, and P. Fuerst. 1983. An approach to population theory for genes in mitochondria and chloroplasts, and some results. Genetics, 103: 513–527.

Birky, C.W., P. Fuerst, and T. Maruyama. 1989. Organelle gene diversity under migration, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics, 121: 613–627.

Brodziak, J., D. Bartley, B. Bently, and G. Gall. 1992. Tests of genetic stock identification using coded wire tagged fish. Can. J. Fish. Aquat. Sci. 49: 1507–1517.

Brown, B.L., and R.W. Chapman. 1991. Genetic analysis of American shad entering Chesapeake Bay. Tech. Rep. No. 91-03. Available from Chesapeake Scientific Investigations Foundation, Inc., P.O. Box 11645, Richmond, VA 23230-0045.

Brown, B.L., and K.T. Paynter. 1991. Mitochondrial DNA analysis of natural variation and selectively inbred Chesapeake Bay oysters, *Crassostrea virginica*. Mar. Biol. (Berlin), 110: 343–352.

Brown, W.M., M. George, Jr., and A.C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. 76: 1967–1971.

Carscadden, J.E., and W.C. Leggett. 1975. Meristic differences in spawning populations of American shad, *Alosa sapidissima*: evidence for homing to tributaries in the St. John River, New Brunswick. J. Fish. Res. Board Can. 32: 653–660.

Chakraborty, R. 1992. Multiple alleles and estimation of genetic parameters: computation equations showing involvement of all alleles. Genetics, 130: 231–234.

Chakraborty, R., and P.E. Smouse. 1988. Recombination of haplotypes leads to biased estimates of admixture proportions in human populations. Proc. Natl. Acad. Sci. U.S.A. 85: 3071–3074.

Chapman, R.W., J. Stephens, R.A. Lansman, and J.C. Avise. 1982. Models of mitochondrial DNA transmission genetics and evolution in higher eukaryotes. Genet. Res. 40: 41–57.

Clark, A.G. 1988. Deterministic theory of heteroplasmy. Evolution, 42: 621–626.

Davidson, W.S, T.P. Birt, and J.M. Green. 1989. A review of genetic variation in Atlantic salmon, *Salmo salar* L., and its importance for stock identification, enhancement programmes and aquaculture. J. Fish Biol. 34: 547–560.

Doyle, J.J., and J.L. Doyle. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. *In* Phytochemical bulletin. Vol. 19. Botanical Society of America, Irvine, Calif. pp. 11–15.

Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131: 479–491.

Fournier, D.A., T.D. Beacham, B.E. Riddell, and C.A. Busack. 1984. Estimating stock composition in mixed-stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41: 400–408.

Fraidenburg, M.E., and R.H. Lincoln. 1985. Wild chinook salmon management: an international conservation challenge. N. Am. J. Fish. Manage. 5: 311–329.

Goldstein, D.B., A.R. Linares, L.L. Cavalli-Sforza, and M.W. Feldman. 1995. An evaluation of genetic distances for use with microsatellite loci. Genetics, 139: 463–471.

Grove, T.L., T.J. Berggren, and D.A. Powers. 1976. The use of innate tags to separate spawning stocks of striped bass, *Morone saxatilis*. *In* Estuarine Processes: Proceedings of the 3rd International Estuarine Research Conference, Galveston, Tex., Oct. 7–9, 1975. Vol. 1. *Edited by* M. Wiley. Academic Press, New York. pp. 166–176.

Gyllensten, U. 1985. The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. J. Fish Biol. 26: 691–699.

Hebert, P.D., R.D. Ward, and L.J. Weider. 1988. Clonal-diversity patterns and breeding-system variation in *Daphnia pulex*, an asexual–sexual complex. Evolution, 42: 147–159.

Johnson, J.K. 1990. Regional overview of coded wire tagging of anadromous salmon and steelhead in Northwest America. Am. Fish. Soc. Spec. Publ. 7: 782–816.

Lachenbruch, P.A. 1967. An almost unbiased method of obtaining confidence intervals for the probability of misclassification in discriminant analysis. Biometrics, 23: 639–645.

Lachenbruch, P.A. 1975. Discriminant analysis. Hafner Press, New York.

Leggett, W.C., and J.E. Carscadden. 1978. Latitudinal variation in reproductive characteristics of American shad (*Alosa sapidissima*): evidence for population specific life history strategies in fish. J. Fish. Res. Board Can. 35: 1469–1478.

Long, J.C. 1991. The genetic structure of admixed populations. Genetics, 127: 417–428.

Long, J.C., and P.E. Smouse. 1983. Intertribal gene flow between the Ye'cuana and Yanomama: genetic analysis of an admixed village. Am. J. Phys. Anthropol. 61: 411–422.

Lynch, M., and T.J. Crease. 1990. The analysis of population survey data on DNA sequence variation. Mol. Biol. Evol. 7: 377–394.

Mansuetti, R., and H. Kolb. 1953. A historical view of the shad fisheries of North America. Md. Dep. Res. Educ. Chesapeake Lab. Publ. No. 97.

Marsden, J.E., C.C. Krueger, and B. May. 1989. Identification of parental origins of naturally produced lake trout in Lake Ontario: application of mixed-stock analysis to a second generation. N. Am. J. Fish. Manag. 9: 257–268.

Melvin, G.D., M.J. Dadswell, and J.D. Martin. 1986. Fidelity of American shad, *Alosa sapidissima* (Clupeidae), to its river of previous spawning. Can. J. Fish. Aquat. Sci. 43: 640–646.

Millar, R.B. 1987. Maximum likelihood estimation of mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 44: 583–590.

Milner, G.B., D.J. Teel, F.M. Utter, and G.A. Winans. 1985. A genetic method of stock identification in mixed populations of Pacific salmon, *Oncorhynchus* spp. Mar. Fish. Rev. 47: 1–8.

Morgan, R.P., T.S.Y. Koo, and G.E. Krantz. 1973. Electrophoretic determination of populations of the striped bass, *Morone saxatilis*, in the Upper Chesapeake Bay. Trans. Am. Fish. Soc. 102: 21–32.

Moritz, C., T.E. Dowling, and W.M. Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annu. Rev. Ecol. Syst. 18: 269–292.

Neel, J.V. 1978. Rare variants, private polymorphisms, and locus heterozygosity in Amerindian populations. Am. J. Hum. Genet. 30: 465–490.

Nolan, K., I.I. Wirgin, and J. Grossfield. 1991. Discrimination among Atlantic coast populations of American shad (*Alosa sapidissima*) using mitochondrial DNA. Can. J. Fish. Aquat. Sci. 48: 1724–1734.

Pella, J.J., and G.B. Milner. 1987. Use of genetic marks in stock composition analysis. *In* Population genetics and fishery management. *Edited by* N. Ryman and F. Utter. University of Washington Press, Seattle, Wash. pp. 247–276.

Roberts, D.F., and R.W. Hiorns. 1962. The dynamics of racial admixture. Am. J. Hum. Genet. 14: 261–277.

Roberts, D.F., and R.W. Hiorns. 1965. Methods of analysis of the genetic constitution of a hybrid population. Hum. Biol. 37: 38–43.

Ryman, N. 1983. Patterns of distribution of biochemical genetic variation in salmonids: differences between species. Aquaculture, 33: 1–21.

Schweigert, J.F., and R.E. Withler. 1990. Genetic differentiation of Pacific herring based on enzyme electrophoresis and mitochondrial DNA analysis. Am. Fish. Soc. Spec. Publ. 7: 459–469.

Scott, M.G., and W.B. Scott. 1988. Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. No. 219.

Setzler, E.M., W.R. Boynton, K.V. Wood, H.H. Zion, L. Lubbers, N.K. Mountford, P. Frere, L. Tucker, and J.A. Mihursky. 1980. Synopsis of biological data on striped bass, *Morone saxatilis*. NOAA Tech. Rep. NMFS Circ. No. 433. FAO Synopsis No. 121. pp. 1–69.

Shaklee, J.B., S.R. Phelps, and J. Salini. 1990. Analysis of fish stock structure and mixed stock fisheries by the electrophoretic characterization of allelic isozymes. *In* Electrophoretic and isoelectric focusing techniques in fisheries management. *Edited by* D.H. Whitmore. CRC Press, Inc., Boca Raton, Fla. pp. 173–196.

Shoubridge, E.A. 1978. Genetic and reproductive variance in American shad. M.Sc. thesis, McGill University, Montreal, Quebec, Canada.

Sidell, B.D.E, R.G. Otto, D.A. Powers, M. Karweit, and J. Smith. 1980. Apparent genetic homogeneity of spawning striped bass in the Upper Chesapeake Bay. Trans. Am. Fish. Soc. 109: 99–107.

Sismour, E.N., and R.S. Birdsong. 1986. A biochemical and genetic analysis of American shad (*Alosa sapidissima*)

migrating into Chesapeake Bay. International Symposium on Common Strategies of Anadromous and Catadromous Fishes. Am. Fish. Soc. Spec. Publ. 1: 555.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics, 139: 457–462.

Smouse, P.E., R.S. Spielman, and M.H. Park. 1982. Multiple-locus allocation of individuals to groups as a function of the genetic variation within and differences among human populations. Am. J. Hum. Genet. 119: 445–463.

Smouse, P.E., R.S. Waples, and J.A. Tworek. 1990. A genetic mixture analysis for use with incomplete source population data. Can. J. Fish. Aquat. Sci. 47: 620–634.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503–517.

Stahl, G. 1987. Genetic population structure of Atlantic salmon. *In* Population genetics and the application to fisheries management. *Edited by* N. Ryman and F. Utter. University of Washington Press, Seattle, Wash. pp. 121–140.

Takahata, N., and J. Maruyama. 1981. A mathematical model of extranuclear genes and the genetic variability maintained in a finite population. Genet. Res. 37: 291–302.

Utter, F., and N. Ryman. 1993. Genetic markers and mixed stock fisheries. Fisheries, 18: 11–21.

Utter, F.M., H.O. Hodgins, F.W. Allendorf, A.G. Johnson, and J. Mighell. 1973. Biochemical variants in Pacific salmon and rainbow trout: their inheritance and application in populations studies. *In* Genetics and mutagenesis of fish. *Edited by* J.H. Schroder. Springer-Verlag, Berlin. pp. 329–339.

Wahlund, S. 1928. Zusammensetzung von Population und Korrelations Erscheinunger vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas, 11: 65–106.

Wirgin, I., P. Silverstein, and J. Grossfield. 1990. Restriction endonuclease analysis of striped bass mitochondrial DNA: the Atlantic coastal migratory stock. Am. Fish. Soc. Spec. Publ. 7: 475–491.

Wirgin, I., L. Maceda, J.R. Waldman, and R.N. Crittenden. 1993. Use of mitochondrial DNA polymorphisms to estimate the relative contributions of the Hudson River and Chesapeake Bay striped bass stocks to the mixed fishery on the Atlantic Coast. Trans. Am. Fish. Soc. 122: 669–685.

Wood, C.C., S. McKinnell, T.J. Mulligan, and D.A. Fournier. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. 44: 866–881.

Xu, S., C.J. Kobak, and P.E. Smouse. 1994. Constrained least squares estimation of mixed population stock composition from mtDNA haplotype frequency data. Can. J. Fish. Aquat. Sci. 51: 417–425.

Zouros, E., K.R. Freeman, A.O. Ball, and G.H. Pogson. 1992. Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel *Mytilus*. Nature (London), 359: 412–414.

Vitamin A stores, teratogenesis, and EROD activity in white sucker, *Catostomus commersoni*, from Rivière des Prairies near Montréal and a reference site

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Abstract: White sucker, *Catostomus commersoni*, were sampled from the Rivière des Prairies in the vicinity of Montréal and the upper reaches of the Ottawa River in La Verendrye Park (reference site). Rivière des Prairies fish had lower liver retinol ($p \leq 0.0001$) and retinyl palmitate ($p \leq 0.0001$) concentrations than fish from the reference site. Rivière des Prairies females and males contained only 9.3 and 30%, respectively, of the vitamin A stores detected in reference fish. Age was an important variable. Covariance analysis revealed that the the rate of retinyl palmitate storage with age was higher ($p \leq 0.0001$) for sucker at the reference site. Retinaldehyde was the major retinoid in eggs; however, concentrations were not different between the two sites. The prevalence of malformations was 1.49% among Rivière des Prairies larvae, greater ($p \leq 0.002$) than that of the reference group. Eye deformities (i.e., anophthalmia, synopsia) were 11.8 times more prevalent in the Rivière des Prairies larvae. Ethoxyresorufin-*O*-deethylase (EROD) was greater ($p \leq 0.0003$) in the Rivière des Prairies fish. Liver EROD activity in adult females was positively correlated ($R = 0.81$; $p \leq 0.0004$) with the prevalence of deformities of their progeny. Semiquantitative results showed that embryo mortality also tended to increase with EROD activity.

Résumé : Nous avons comparé les réserves hépatiques et ovariennes de vitamine A entre deux populations de Meunier noirs (*Catostomus commersoni*), capturé sur leur site de fraie, soit dans la Rivière des Prairies à proximité de Montréal et dans la partie amont de la Rivière des Outaouais (site référence). Les Meuniers noirs de la Rivière des Prairies avaient moins de rétinol ($p \leq 0{,}0001$) et de palmitate de rétinol ($p \leq 0{,}0001$) hépatique que les meuniers du site référence. Les concentrations de la vitamine A mesurées dans les femelles et les mâles de la Rivière des Prairies représentaient seulement 9,3 et 30% des concentrations mesurées pour les femelles et mâles, respectivement, du site référence. L'âge est une covariable importante. Une analyse de covariance a montré que le taux d'entreposage annuel du palmitate de rétinol dans le foie est plus grand ($p \leq 0{,}0001$) pour les meuniers du site référence. Les concentrations ovariennes de rétinoïdes n'étaient pas différentes entre les deux sites. Le rétinal est la principale forme entreposée dans les oeufs. La prévalence des malformations des larves de la Rivière des Prairies était plus élevée (1,49%; $p \leq 0{,}002$). Les malformations de l'oeil (e.g., anophthalmie, synopsie) étaient 11,8 fois plus élevées dans les larves de la Rivière des Prairies. L'activité enzymatique éthoxyrésorufine-*O*-dééthylase (EROD) du foie était plus élevée ($p \leq 0{,}0003$) pour les meuniers capturés à la Rivière des Prairies. Nous avons trouvé une corrélation positive ($R = 0{,}81$ $p \leq 0{,}0004$) entre l'activité EROD hépatique des femelles et le pourcentage de malformations de leur F_1 respective. De façon semi-quantitative, la mortalité des embryons aussi augmentait avec l'activité EROD.

Received May 13, 1993. Accepted March 2, 1995.
J11930

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Introduction

The term vitamin A refers to compounds such as retinol, retinoic acid, and other structurally related molecules (e.g., dehydroretinol) having characteristic biological activities. These retinoids and their carotenoid precursors are not synthesized de novo by vertebrate animals, but originate in plants and are transferred through food webs. Fish are capable of converting different carotenoids into biologically active retinoids (Barua and Goswami 1977; Schiedt et al. 1985). Excess retinoids are usually esterified to inactive forms (e.g., retinyl palmitate, dehydroretinyl palmitate) that are stored in various organs such as the liver and may be mobilized depending upon dietary vitamin A intake and physiological requirements.

Whereas vitamin A has been researched in some detail in birds and mammals, only one publication pertains to its essential role in teleosts. Feeding diets deficient in vitamin A to brook trout (*Salvelinus fontinalis*) "swim-up" larvae (the stage at which exogenous food is first ingested) resulted in growth rate reduction, a ventrolateral swelling associated with peritoneal effusion (i.e., ascites), dermal depigmentation, and ocular lesions (Poston et al. 1977). These symptoms were either reversed or prevented by supplementing the experimental diet with either retinyl palmitate or high levels of β-carotene. Many other symptoms of vitamin A deficiency have been identified in birds and mammals and the manifestation of deficiency symptoms is associated with low (or depleted) liver retinoid stores and a decline in blood retinol concentration (Thompson 1970; Underwood 1984). Precise regulation of intracellular retinoic acid concentrations is apparently necessary for normal embryonic development, as slight changes (either increases or decreases) may cause deformities (Thompson et al. 1969; Tickle et al. 1985; Thaller and Eichele 1987). Additionally, the level of carotenoids in fish eggs is positively correlated with percent viability (Mikulin and Soin 1975; Tacon 1981).

Environmental contaminants such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) decrease liver vitamin A stores in mammals (Phillips 1963; Bitman et al. 1972; Thunberg et al. 1979). We previously reported that rats exposed to a coplanar biphenyl congener demonstrated an accelerated rate of retinoic acid conversion to 4-hydroxy-, 4-oxo-, and β-glucuronide metabolites consistent with enzyme induction mechanisms (Spear et al. 1988). A coplanar biphenyl decreased liver and egg yolk retinoids in laboratory studies with birds (Spear et al. 1986, 1989). Extremely low retinoid stores in the livers of wild birds on the Great Lakes were inversely related to TCDD levels, and presumably a multitude of other contaminants (Spear et al. 1986, 1992). Also in the case of wild birds, changes in egg yolk retinoids have been correlated with TCDD toxic equivalents for Great Lakes colonies (Spear et al. 1990) and correlated with specific PCB congeners for St. Lawrence River colonies (Boily et al. 1994). Parallel to these relationships between environmental contaminants and vitamin A, reproductive failure and a high prevalence of malformations have been reported in Great Lakes bird colonies since the 1960s and continue to be a major problem in sensitive piscivorous species (Hoffman et al. 1987; Gilbertson et al. 1991).

Reproductive failure and a high prevalence of malformations were also reported for fish populations in contaminated areas (Bengtsson et al. 1985; Karås et al. 1991; Mac and Edsall 1991; Lindesjöö and Thulin 1992). The prevalence of malformations in noncontaminated waters varies from 0.06 to 0.26% (Berra and Au 1981) whereas Bengtsson et al. (1985) listed prevalences ranging from 1 to 60% for polluted areas of Sweden.

In the course of other investigations, we accidentally discovered deformities in white sucker, *Catostomus commersoni*, captured near Montréal. The present study was designed to quantify the prevalence of malformations in white sucker embryos under optimal developmental conditions in the laboratory. Embryos of fish captured near Montréal were raised to the beginning of the larval stage (swim-up) under controlled conditions to exclude extrinsic variables (e.g., temperature, dissolved oxygen, waterborne toxicants) that may cause significant malformations. In this way, factors intrinsic to the egg and embryo (e.g., genetic factors, nutrients contained in the vitellus, accumulated toxic substances) could be studied. Embryos from fish collected at a reference site were also raised under the same laboratory conditions, which permitted us to discern whether the prevalence at the Montréal site was, in fact, elevated as our casual observations had indicated.

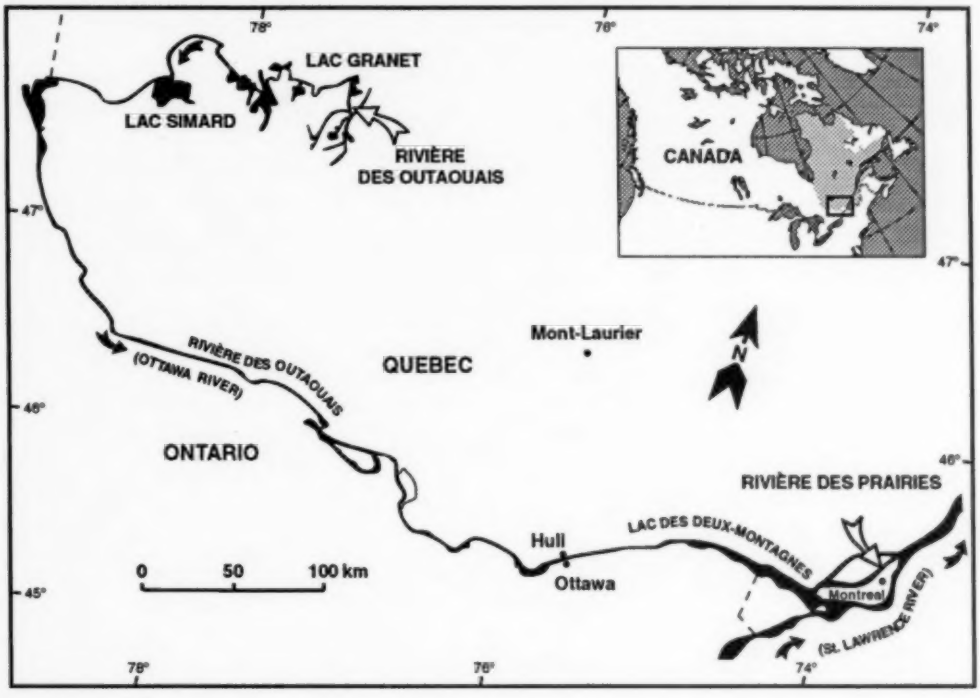
Two study sites were selected on the same river system. The sites are separated by a series of dams and natural barriers over a distance of 1000 km, preventing movement of adults between sites. The reference site, near Lac Granet at the origin of the Ottawa River (Rivière des Outaouais), is located in a wildlife reserve distant from contaminant emissions (Fig. 1). Suckers caught at the Montréal site are potentially exposed to a mixture of contaminants and do not have access to a pristine site during their life cycle. The essential role of retinoids in supporting reproduction and especially the morphogenic activity of retinoic acid (Thompson 1970; Thaller and Eichele 1987; Ganguly 1989) led us to the hypothesis that the vitamin A status of white sucker is different between the two study sites. Therefore, retinoids and β-carotene were analyzed in both egg and adult liver samples. The activity of ethoxyresorufin-*O*-deethylase (EROD) was assayed in adult liver microsomes as it is generally accepted as a biomarker for exposure to contaminants inducing cytochrome P-450IA (e.g., polyhalogenated dibenzo-*p*-dioxins, dibenzofurans, and coplanar PCBs, in addition to polycyclic aromatic hydrocarbons (PAHs)) and because hydroxylation through cytochrome P-450 is known to be a step in the retinoid metabolic pathway. To our knowledge, this is the first study of the possible effects of contaminants on the vitamin A status of natural fish populations.

Materials and methods

Study sites

The reference site was located on the Ottawa River near its origin in La Verendrye Park (Fig. 1). A survey of contaminants in fish from this area revealed low levels of organochlorines. PCB concentrations were below the limit of detection (0.5 $\mu g \cdot kg^{-1}$) in Lake Simard (Paul and Laliberté 1988). The Rivière des Prairies site was located

Fig. 1. Map showing the reference spawning site on the Rivière des Outaouais and the Rivière des Prairies spawning site near Montréal.



about 1000 km downstream from the reference site. The Ottawa River receives various types of municipal and industrial wastes before entering the Lac des Deux Montagnes. The Rivière des Prairies drains the Lac des Deux Montagnes and flows around the northern side of Montreal Island before joining the St. Lawrence River. Fish from this region have moderate to high levels of PCBs, PAHs, and heavy metals (Paul and Laliberté 1989; Goulet and Laliberté 1982).

Fish

Approximately 80 adult white sucker were captured either with gill nets or by electrofishing on their spawning grounds at each site in early May 1990 when the water temperature reached 9–10°C. The fish were held in enclosures at the site of capture. Ten individuals of each sex were chosen randomly from the enclosures at each site. Fish were weighed (±10 g) by using a suspended spring balance. Total length was measured to the nearest millimetre. Age was determined by counting annuli on the opercular bone under a dissecting microscope. Two-way analysis of variance (ANOVA) revealed significant ($p \leq 0.0001$) differences in weight and total length between sexes; however, no differences were apparent between sites with respect to age, weight, or total length.

Teratogenesis

Eggs and milt were expelled by applying slight pressure to the abdomen. The roe of each female was fertilized separately by dry fertilization. To maintain a high degree of genetic variability, a minimum of two males was used as milt donors for the eggs of each female. Five minutes after milt activation by contact with water, the eggs were thoroughly rinsed with river water, placed in plastic bags, and allowed to water harden for at least 1 h before being transported to the laboratory. At the Rivière des Prairies, only 7 females of about 40 were running ripe. In contrast, all females at the reference site were running ripe. A total of 200 mL of eggs from each female was incubated individually at 13.5°C in two suspended baskets (Nitex mesh, 500 μm) placed randomly in an artificial stream of 600-L capacity supplied with degassed, dechlorinated, and reoxygenated water at a rate of 2.5 $L \cdot min^{-1}$. Two artificial streams were used, one for each site. A revolving paddle mechanism maintained a slight water current. Photoperiod was set at 14 h light : 10 h dark. Only diffuse, low-intensity light reached the eggs.

Development was terminated 17 days after fertilization, just before the start of exogenous feeding when yolk sac absorption is almost complete (beginning of the swim-up stage). Thus, it was possible to investigate maternal (endogenous) factors influencing development and to correlate other biochemical indicators measured in females with their respective F_1 success. Embryos were preserved in 10% Formalin until examination of gross morphology under a dissecting microscope. A total of 200 embryos per basket (400 per female) were examined with the exception of four Rivière des Prairies females whose progeny had high mortality. The embryos were carefully examined for a wide range of developmental anomalies. All visible

anomalies were compiled and classified according to Sharp's (1991) pictorial atlas. Predominant among these were anophthalmia, synopsia, abdominal edema, scoliosis, supernumerary fins, and heart deformities. Eggs attacked by fungus as well as dead embryos were removed from baskets and were not considered in the estimation of malformation prevalence. No chemical treatment was used to control fungal growth.

Vitamin A analyses

Mature eggs were obtained by stripping (as described above) and were subsampled before fertilization. Adults were killed by cervical severance and a 1-g caudal portion of liver was excised. The egg and liver samples were wrapped in plastic bags to exclude air, placed inside a plastic vial, and immediately plunged into liquid nitrogen. Thereafter, samples were held on dry ice for transport and preserved at −80°C until analysis.

Retinoids were extracted and analyzed under subdued, incandescent, yellow light to prevent isomerization. Egg retinoids were extracted according to the method of Spear and Moon (1986) with the exception that the hexane–tissue mixture was kept for 24 h in complete darkness at 4°C for equilibration before the final stage of extraction (Olson 1979).

Liver retinoids were extracted and analyzed by the isocratic, reverse-phase high-performance liquid chromatography (HPLC) method of Spear and Moon (1986), using a model 590 pump, a model 441 absorbance detector set at 340 nm, and a model U6K manual injector (all components from Waters Assoc., Milford, Mass). The stationary phase consisted of a 25 cm × 4.6 mm C18 μBondapack column with 10-μm particle size and the mobile phase was methanol–water (96:4). Hepatic β-carotene was analyzed on a Beckman Instruments (Montréal, Quebec) reverse-phase HPLC system consisting of a model 210A injector equipped with a 100-μL loop, a model 126 pump, and a 168 diode array detector with continuous monitoring at 453 nm. Liver β-carotene was eluted within 20 min isocratically using an acetonitrile–chloroform (90:10) mobile phase and a 25 cm × 4.6 mm, 10 μm particle size, C18 μBondapack column. Retinoids and β-carotene in egg extracts were separated by a gradient reverse-phase method (Furr 1990) using a 15 cm × 4.6 mm Ultrasphere, 5 μm particle size, C18 column (Beckman Instruments, Montréal, Quebec).

All-*trans*-retinol, all-*trans*-retinal, all-*trans*-retinyl palmitate, and β-carotene standards were purchased from Sigma Chemical Co. (St. Louis, Mo.). All-*trans*-3,4-didehydroretinol and all-*trans*-3,4-didehydroretinyl palmitate were synthesized in our laboratory according to methods outlined in Barua and Olson (1984).

EROD assay

NADPH, 7-ethoxyresorufin, and 7-hydroxyresorufin were obtained from Sigma. Liver samples were collected and preserved as described above. All steps of microsome preparation were performed at 0–4°C and the reaction was conducted at 15°C, which approximates the water temperature at the time of capture. Tissue was homogenized with a motor-driven Potter–Elvehjem in 3 volumes of Tris-HCl buffer (pH 7.4). The S_{10} fraction was centrifuged at $10^5 \times g$ for 1 h and microsomes were resuspended in 2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid (HEPES) buffer (pH 7.5). Specific EROD activity was estimated by direct fluorometric detection (Perkin–Elmer model LS 50 spectrofluorometer) of the 7-hydroxyresorufin metabolite as reported by Prough et al. (1978). Following a 4-min preincubation at 15°C, the reaction was started by addition of NADPH. The linear phase of preincubation fluorescence change was subtracted from the linear (initial) phase of reaction fluorescence change to correct for any nonspecific reaction. Microsomal protein was measured with Folin phenol reagent (Lowry et al. 1951) using bovine serum albumin (BSA) as standard.

Statistical analysis

To estimate the prevalence of malformations for each site, the number of malformations was divided by the total number of larvae examined at each site. The difference in prevalence between sites was assessed by the chi-square test. Hepatic retinoid and β-carotene concentrations as well as EROD activities were evaluated using two-way ANOVAs with site and sex as the two classes. The variables were log transformed to respect the assumptions of ANOVA. Differences in egg retinoids between sites were tested by a one-way ANOVA. The rate of storage of retinyl palmitate with age between sites and the retinol – retinyl palmitate relationship between sexes were tested using analysis of covariance (ANCOVA). A correlation matrix between all variables was computed and Pearson coefficients were double checked with the Bonferroni correction. Statistical analysis were performed using the SAS package (SAS Institute, Inc., Cary, N.C.) on an IBM mainframe computer.

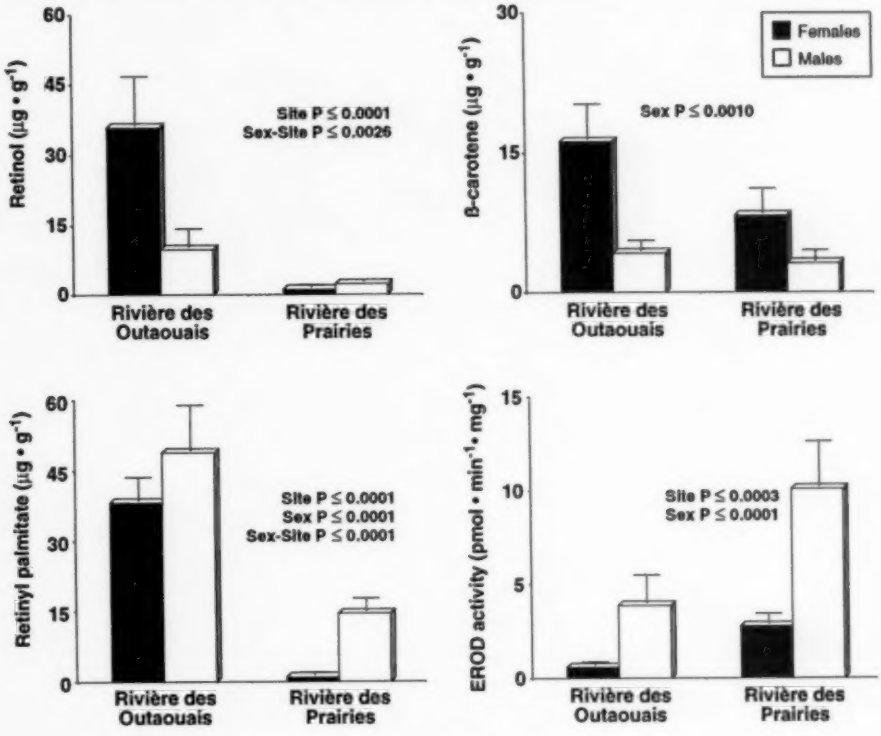
Results

Retinoids and β-carotene

The analyses of liver samples by HPLC revealed that the white sucker normally stores retinoids as all-*trans*-retinyl palmitate with lesser amounts of all-*trans*-3,4-didehydroretinyl palmitate and other unidentified esters. Similarly, all-*trans*-retinol was found to be the predominant unesterified form in the liver whereas all-*trans*-3,4-didehydroretinol was infrequently detected. These results indicated that the white sucker is a retinol-type (vitamin A_1) species according to the classification of Goswami and Barua (1981). Despite the fact that several retinyl esters were detected, their proportions relative to all-*trans*-retinyl palmitate were quite consistent between individual fish. Therefore, all-*trans*-retinyl palmitate was quantified to represent the esterified forms.

Hepatic concentrations of all-*trans*-retinol (retinol) and all-*trans*-retinyl palmitate (retinyl palmitate) differed significantly ($p \leq 0.0001$) between sites (Fig. 2). White sucker captured at the reference site contained 13.4 and 5.7 times more retinol and retinyl palmitate stores, respectively, than those from the Rivière des Prairies site. The two-way ANOVA also revealed a significant sex effect ($p \leq 0.0001$) for retinyl palmitate, with males having greater concentrations than females. In the case of retinol, the sex × site interaction was significant ($p \leq 0.0026$), indicating that the site effect was different between males and females. The

Fig. 2. Liver retinol, retinyl palmitate, and β-carotene concentrations as well as liver microsomal EROD activities of adult white suckers collected from the reference and Rivière des Prairies sites. Values are given as means and standard errors.



concentration of retinol in reference females was 31.1 times that in Rivière des Prairies females, whereas the retinol concentration in reference males was 4.3 times greater than in Rivière des Prairies males. The interaction was also significant ($p \leq 0.0001$) for retinyl palmitate (Fig. 2), with reference males having 39.3 times more than Rivière des Prairies males, while reference females contained only 3.4 times the concentration detected in Rivière des Prairies females.

In addition to the variables sex and site, a significant influence of age was identified with respect to the storage of retinyl palmitate in the liver. Older individuals collected at the reference site were found to have increased amounts of stored retinoids according to the following linear regression:

$$[1] \quad P = 13.20 + 2.30A$$

where P is the concentration of retinyl palmitate ($\mu g \cdot g^{-1}$) and A is the age (yr) of individual fish. Equation 1 was significant with respect to slope ($p \leq 0.0187$; $R^2 = 0.27$) whereas the intercept was not different from zero. The influence of age in this case means that the sex-related difference with respect to the reference site (Fig. 2) may actually have been an artifact of age. Males were older (2.5 yr on average) than females at the reference site, and applying a correction factor for age essentially eliminated the disparity in concentrations of retinyl palmitate between males and females (data not shown). Thus, reference males and females stored similar levels of retinyl palmitate when the data were normalized for the influence of age.

With respect to fish from the Rivière des Prairies site, however, the sex-related difference was opposite to what would be expected if age were a major contributing variable. This point is illustrated by the covariance analysis that was performed to evaluate the effect of site on the rate of accumulation of retinyl palmitate in liver tissue (Fig. 3). The general ANCOVA model was significant ($p \leq 0.0001$), with age explaining a significant part of the variation in retinyl palmitate ($R^2 = 0.59$; see eq. 2 below). One should note that the overall effect of age was not significant ($p \leq 0.07$) because of a weak relationship for fish from the Rivière des Prairies, whereas it was significant in the regression model described above for reference fish (eq. 1) and in the following relationship:

$$[2] \quad P = 13.40 \text{ (ns)} + 2.29A \ (p \leq 0.0004) - 2.58D_1A \ (p \leq 0.0001)$$

where P is the concentration of retinyl palmitate ($\mu g \cdot g^{-1}$), A is age (yr), and $D_1 = 0$ for the reference site and $D_1 = 1$ for the Rivière des Prairies site. The effect of site on the intercept was not significant (ns); however, its effect on the slope was ($p \leq 0.0001$). The levels of significance of the differential slopes are given in parentheses. It is clear from Fig. 3 that reference fish progressively increased

Fig. 3. Relationship between liver retinyl palmitate stores and age of adult white sucker. An ANCOVA model demonstrates a significant ($p \leq 0.0001$) effect of site on the slope (see eq. 2 in the text).

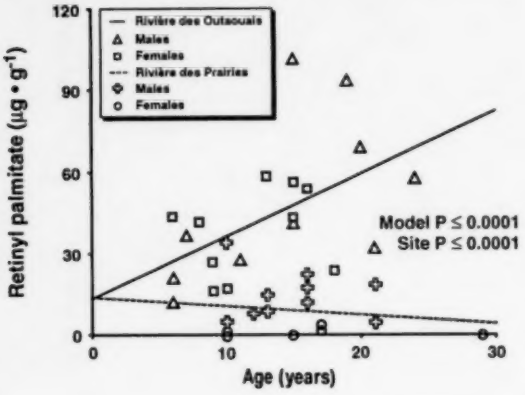
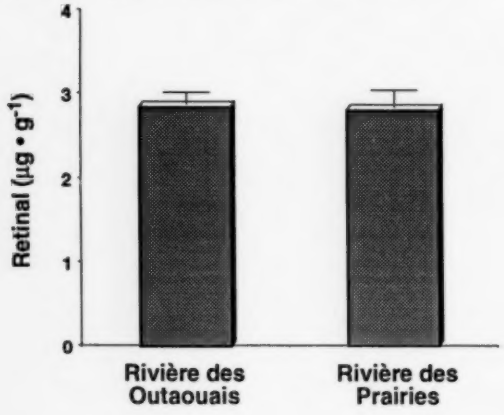


Fig. 4. Retinal concentrations in eggs of white sucker. Values are given as means and standard errors.



their stores of retinyl palmitate as they grew older, in contrast to fish from the Rivière des Prairies that tended to have decreasing hepatic retinyl palmitate stores with age.

Liver retinol concentration increased with retinyl palmitate. The relationship is shown by an ANCOVA model (see eq. 3 below; $p \leq 0.0001$; $R^2 = 0.54$) because the effect of sex on the slope was significant ($p \leq 0.0001$); thus, the ratio of hepatic retinol to hepatic retinyl palmitate was different between the two sexes. The effect of sex on the intercept was not significant. The resulting equation is

$$[3] \quad R = -0.68\ (\text{ns}) + 0.22P\ (p \leq 0.0510) + 0.73D_1P \quad (p \leq 0.0001)$$

where R is the concentration of retinol ($\mu g \cdot g^{-1}$), P is the concentration of retinyl palmitate ($\mu g \cdot g^{-1}$), and $D_1 = 0$ for males and $D_1 = 1$ for females. The levels of significance of intercept differences (ns) and differential slopes are given in parentheses.

Table 1. Vitamin A stores in the liver of white sucker.

	Vitamin A stores		
	Rivière des Outaouais (reference site)	Rivière des Prairies	Percent change compared with reference site (%)
Males	135.4	41.2	30.0
Females	227.9	21.3	9.3
Total	363.3	62.5	17.2

Note: Vitamin A stores are expressed as nanomoles of retinol equivalents per gram of liver. Equivalents were calculated as the sum of the stores (in nanomoles per gram of liver) of retinol plus retinyl palmitate plus β-carotene.

Livers were also analyzed for β-carotene, a metabolic precursor of retinoids (Fig. 2). Females at both sites stored more β-carotene than males ($p \leq 0.0010$) and no differences were found between sites.

To provide an overall picture of vitamin A storage, data were converted to molar equivalents and summed to give a single value at each study site (i.e., millimoles of retinol + millimoles of retinyl palmitate + millimoles of β-carotene). The resulting values (Table 1) demonstrate that the vitamin A stores in females from the Rivière des Prairies were 9.3% of those in females collected from the reference site. The vitamin A stores of Rivière des Prairies males were 30% of those of reference males.

In contrast to their concentrations in the liver, the levels of retinoids in the eggs did not vary greatly between individuals (Fig. 4). All-*trans*-retinal (retinal) was found to be the prevailing egg retinoid. Simple ANOVA revealed no differences between sites for retinal concentration. Small traces of all-*trans*-retinol were detected, but no retinyl esters or β-carotene were apparent. The concentration of retinal stored in eggs before spawning was much lower than the hepatic concentrations of retinol or retinyl palmitate.

EROD activity and teratogenesis

EROD activity was significantly ($p \leq 0.0003$) different between sites (Fig. 2). The difference between sexes was also significant ($p \leq 0.0001$), with males showing higher activity than females. Activities were 4.8 and 2.6 times greater in Rivière des Prairies females and males, respectively, than in fish from the reference site.

The total prevalence of malformations was significantly different between the two sites (chi-square test; $p \leq 0.002$): 0.54% for reference larvae and 1.49% for Rivière des Prairies larvae (Table 2). The overall relative risk factor associated with malformations was 2.8. Eye malformations, such as anophthalmia and synopsia (partial cyclopia), were the most prevalent anomalies observed in larvae having a relative risk factor of 11.8. Abdominal edema was also a common anomaly in larvae of the Rivière des Prairies females (0.50%).

During the summer of 1989, we observed deformities in 5% of adult white sucker (*C. commersoni*) and lake

Table 2. Prevalence of major malformations in white sucker larvae.

Malformation	Prevalence (%)		
	Rivière des Outaouais (reference site)	Rivière des Prairies	Relative risk factor[a]
Eyes	0.042	0.497	11.8
Fins	0.042	0.166	4.0
Edema	0.167	0.497	3.0
Total[b]	0.542	1.490	2.8

[a]Calculated as the prevalence of terata at the Rivière des Prairies site divided by the prevalence at the reference site.

[b]Total prevalence includes other types of malformations than those described. Prevalence differed significantly between sites (chi-square test; $p \leq 0.002$).

sturgeon (*Acipenser fulvescens*) in the vicinity of Montreal Island. Most deformed fish displayed duplication of a pectoral or pelvic fin, and many cases of bifurcated rostrum were also documented. In the present study, the prevalence of fin anomalies (0.17%) was lower than that observed during the preliminary investigation. The rostrum of the larvae in the present study had not assumed the juvenile morphology, and deformities such as bifurcated rostrum could not be assessed.

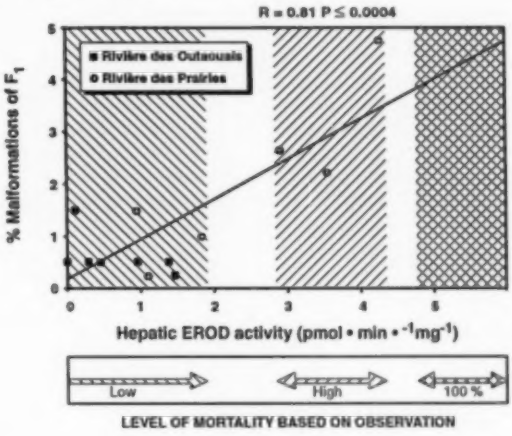
EROD activity of females and the prevalence of malformations in the F_1 generation were correlated (Fig. 5). The probability of this positive correlation is $p \leq 0.0004$ with a Pearson coefficient of $R = 0.81$; the Bonferroni correction for type-1 error $\alpha = 0.05$ was 0.0014. Mortality was estimated indirectly from the number of larvae surviving at the end of the experiment. The following three classes were defined: low (i.e., ≥200 embryos surviving), high (i.e., ≤200 embryos surviving), and 100% mortality. It is interesting to note that the three females with high F_1 mortalities had relatively elevated enzymatic activities (Fig. 5). Moreover, the only female showing 100% mortality in her progeny had the highest level of EROD activity (represented as the cross-hatched area in Fig. 5).

Other correlations are interesting in the context of vitamin A physiology and particularly its role in growth. A positive correlation between total length and age of adults was obtained for reference females ($R = 0.69$; $p \leq 0.0287$) and males ($R = 0.91$; $p \leq 0.0003$) but not in the case of Rivière des Prairies females or males. Although the Bonferroni correction for females was 0.0014 for type 1 error $\alpha = 0.05$, this correlation has a high degree of biological interest and we consider it to be significant. A positive correlation between weight and age was also significant in reference males ($R = 0.96$; $p \leq 0.0287$) but not Rivière des Prairies males.

Discussion

Reproductive strategies vary greatly among taxonomic classes. Fish generally produce a large number of gametes with considerable loss during development such that few

Fig. 5. Correlation between prevalence of malformations of F_1 specimens and the liver microsomal EROD activities of the corresponding females. Relationship to embryo mortality is also presented.



survive to adulthood and complete the life cycle. Embryonic mortality and occasional deformities are thus observed in most fish species (see for instance Dawson 1964, 1966, 1971; Dawson and Heal 1976). In the present study, however, the malformation prevalence of 1.49% among embryos of the Rivière des Prairies fish was relatively large and significantly greater ($p \leq 0.002$) than that estimated for reference embryos. In the case of ocular deformities, the prevalence was 12 times greater in the Rivière des Prairies embryos (Table 2). These results confirm our preliminary observations concerning an elevated prevalence of malformations in white sucker caught in the Rivière des Prairies near Montréal.

Although the prevalence of malformations was significantly greater in the Rivière des Prairies embryos, it was less than the value of 5% estimated in adults in our preliminary observations. This apparent discrepancy may be explained by the fact that deformities observable only in larvae (e.g., duplication of the pelvic fins) were not included because the experiment was terminated before this developmental stage. (The logistics of feeding large numbers of larvae forced us to terminate the experiment at the end of the embryonic stage.) Secondly, the influence of factors extrinsic to the fertilized egg (e.g., temperature, physical disturbances, waterborne contaminants) was minimized by raising the embryos in the laboratory under optimal conditions. In other words, the difference in the prevalence of malformations may have been greater if the eggs had been submitted to environmental stressors normally encountered on a spawning ground. Many contaminants are potentially teratogenic to fish embryos by direct exposure (Rosenthal and Alderdice 1976; Weis and Weis 1977; Mauck et al. 1978; Helder 1980). The elimination of such extrinsic factors from the present study may have further reduced the estimate of prevalence from that occurring naturally in the Rivière des Prairies. The design of the present experiment, therefore, provides a conservative estimate of the true prevalence.

The difference in malformation prevalence between the two study sites is attributed to intrinsic factors (e.g., genetics and heredity, vitellus quality, accumulated contaminants). So far, we have analyzed subsamples of the eggs for accumulated levels of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans that were both below limits of detection (Dr. Patrick O'Keefe, Wadsworth Center for Laboratories and Research, New York State Department of Health, unpublished results). The sum of coplanar PCBs in subsamples of the white sucker eggs was found to be 28.4 $ng \cdot g^{-1}$ in the Rivière des Prairies group, which was 36 times greater ($p \leq 0.002$) than that in the reference group (P.A. Spear, C.-S. Hong, A. Branchaud, T. Houck, and B. Bush, unpublished results). These results are consistent with the possibility that toxic contaminants transferred from the adult female to her eggs may have contributed to the elevated prevalence of malformations. Niimi (1983) reported the transfer of organic contaminants and mercury to white sucker eggs. Similarly, embryo mortality has been associated with the transfer of contaminants from adult females to their eggs (Hogan and Brauhn 1975; von Westernhagen et al. 1981; Hansen et al. 1985; Mac et al. 1985; Westin et al. 1985; Gillepsie and Baumann 1986; Spies and Rice 1988; Mac and Edsall 1991). A comprehensive investigation of other intrinsic factors has not been conducted and these factors may also have contributed to developmental anomalies.

EROD is a biomarker for exposure to specific groups of environmental contaminants: polyhalogenated dibenzo-*p*-dioxins, dibenzofurans, and coplanar PCBs, as well as PAHs and other chemicals that induce cytochrome P-4501A (Hodson et al. 1991). The results pertaining to EROD (Fig. 2) are consistent with the possibility that the Rivière des Prairies sucker experienced a greater exposure to coplanar PCBs or PAHs than the reference fish. The correlation between prevalence of malformations and hepatic EROD activity (Fig. 5) suggests that those adult females exposed to higher levels of contaminants that induce cytochrome P-4501A produced eggs more likely to develop abnormally. Spies and Rice (1988) also demonstrated relationships between development and a cytochrome P-4501A biomarker. In their study, percent viable eggs, fertility, and embryo survival were negatively correlated with hepatic aryl hydrocarbon hydroxylase activity, whereas embryological success was negatively correlated with PCB concentrations in eggs. Although it was not the purpose of the present study to examine embryo mortality in detail, Fig. 5 depicts a tendency toward increasing embryo mortality in eggs produced by females with higher EROD activities. These findings suggest a possible relationship between exposure and reproductive success in white sucker.

The absolute values of EROD activity are lower than those normally presented in the literature. This is presumably due to the lower temperature of our assay and also to the long storage period of whole liver tissue before the activity was measured (26 months). Also, EROD was presumably suppressed by steroid interactions associated with spawning (Hodson et al. 1991).

The difference in hepatic vitamin A concentration between study sites (Fig. 2) is consistent with results previously obtained for contaminated populations of wild birds in the Great Lakes (Spear et al. 1986, 1992). That is, white sucker inhabiting the Rivière des Prairies contained extremely low levels of liver retinoid stores as did herring gulls, *Larus argentatus*, on Lake Ontario. In the case of Great Lakes gulls, liver retinoid stores were inversely related to TCDD accumulation (and presumably other contaminants). An inverse relationship was demonstrated between gull liver retinol stores and the activity of a cytochrome P-4501 dependent enzyme, aryl hydrocarbon hydroxylase (Spear et al. 1992). Decreased liver retinoid stores were reported in lake trout, *Salvelinus namaycush*, following exposure to 3,3′,4,4′,5-pentachlorobiphenyl, a coplanar biphenyl (Palace and Brown 1994).

Equation 1 clearly shows that sucker at the reference site increase their liver retinoid stores with age. The difference in slope between sites (eq. 2) means that the rate of accumulation of retinyl palmitate for the Rivière des Prairies fish is different from that for the reference fish. In fact, eq. 2 reveals that liver retinyl palmitate levels in the Rivière des Prairies sucker decreased with age, which may mean that the fish lose the retinoids they were able to store in their first years of life. Differences in vitamin A stores, therefore, should be readily apparent in older fish or species with a long life-span, whereas sampling younger fish may not reveal such differences. Liver vitamin A may be a time-integrated measure of effects during chronic exposure to environmental contaminants.

The significant interactions in the two-way ANOVA performed for liver retinol and retinyl palmitate (Fig. 2) underline the finding that the Rivière des Prairies females contained relatively less retinoid stores than would be expected on the basis of differences between study sites or differences between sexes. The retinol – retinyl palmitate relationship gave a coefficient of 0.95 (eq. 3) for females, meaning that the concentrations of the two retinoids were similar, which may reflect high prespawning levels of retinol mobilized for egg formation. In mammals, hepatic retinol normally represents only a small proportion (5%) of what is stored as retinyl esters (Ganguly 1989). The coefficient for males of 0.22 (eq. 3) means that the concentration of retinol was considerably less than that of retinyl palmitate.

The lower retinoid stores in Rivière des Prairies white sucker may be explained by several possible mechanisms. The large geographical differences between the sites mean that a host of factors (e.g., annual variations in water temperature, overwintering, food availability, food quality, primary productivity, density) may explain the differences in retinoid stores. Perhaps the most obvious is lower vitamin A levels in the diets of Rivière des Prairies fish; however, hepatic β-carotene concentrations were not different between sites as would be expected if nutritional availability were the principal mechanism. β-Carotene can normally be converted to retinoids in the liver and unless its metabolism were inhibited, it would contribute to liver retinoid stores.

A second possible mechanism is metabolic degradation of liver retinoid stores. Enzymes inducible by toxic substances are known to be involved in retinoid metabolism in the liver (Roberts et al. 1980; Frolik 1984; Blaner and

Olson 1994). Accelerated hydroxylation of retinoic acid by the cytochrome P-450 system and conjugation by UDP-glucuronyltransferase were demonstrated in rats injected with 3,3′,4,4′,5,5′-hexabromobiphenyl, a coplanar biphenyl (Spear et al. 1988). In the case of rainbow trout, *Oncorhynchus mykiss*, injected with a coplanar biphenyl, the rate of retinoic acid hydroxylation was significantly increased (Gilbert et al. 1995). The rate of conversion of retinol to retinal increased after treatment of rats with 3-methylcholanthrene (Shih and Hill 1991).

A third possible explanation relates to stellate cells that are known to store large amounts of retinoids in mammals. Stellate cells have been identified in the liver of several fish species (Wake et al. 1986). Mechanisms such as necrotic loss and transformation of stellate cells have been suggested by Chen et al. (1992) to explain the effect of PCBs, polybrominated biphenyls (PBBs), and TCDD on the dynamics of vitamin A in the liver of rat. These, or other mechanisms involving vitamin A storage, may have occurred in white sucker in the present study.

The absence of a correlation between length and age of Rivière des Prairies spawning adults of both sexes, in addition to the absence of a correlation between weight and age for Rivière des Prairies males, may be related to a growth effect. Vitamin A deficiency is associated with growth inhibition (Poston et al. 1977; Ganguly 1989). Depressed growth after sexual maturity has also been reported in studies of contaminated fish populations (Munkittrick and Dixon 1988, 1989; McMaster et al. 1991).

Although marked differences were found in the liver concentrations of vitamin A between study sites, no differences were observed with respect to vitamin A stores in eggs. We found no evidence in the present study to support the hypothesis that the retinoid status in ovo is related to the increased prevalence of terata in the Rivière des Prairies fish. However, techniques are not presently available that would permit the study of endogenous concentrations of the known morphogen, retinoic acid, in fish eggs. The absence of β-carotene in eggs is consistent with other reports showing that fish selectively accumulate xanthophyll as a major carotenoid (Mikulin and Soin 1975; Tacon 1981).

The results of the egg retinoid analysis do not concur with our previous observations in birds. In the case of herring gull eggs collected from the Great Lakes, the ratio of retinol to retinyl palmitate in the vitellus was positively correlated with contamination by polychlorinated dibenzo-*p*-dioxins and dibenzofurans (Spear et al. 1990). In eggs of the ring dove injected with a coplanar biphenyl congener, the ratio of retinol to retinyl palmitate and blood retinol levels suggested an effect of the contaminant on oogenesis (Spear et al. 1989). Retinyl palmitate concentrations in the eggs of great blue herons sampled at colonies along the St. Lawrence River were negatively correlated with accumulated levels of coplanar PCBs and certain other PCB congeners (Boily et al. 1994). Perhaps the vitellogenic process in birds leading to the incorporation of retinoids in the vitellus is more sensitive to the effects of such organochlorine compounds than it is in fish.

An additional finding suggests that the reproduction of Rivière des Prairies suckers is inhibited. We captured approximately 40 adult females near the spawning site on the Rivière des Prairies; however, only 7 were running-ripe (see Materials and methods). All females caught at the reference site were running ripe. McFarlane and Franzin (1978) reported reduced egg diameter and egg retention in 50% of white sucker females in a lake polluted by various heavy metals. In white suckers exposed to bleached kraft pulp mill effluents, the pituitary–gonadal axis was disrupted at multiple sites leading to nonresponsiveness of females to artificial induction of spawning with gonadotropin (Van Der Kraak et al. 1992). Similar observations of failure in artificial induction of spawning were made on females of the copper redhorse, *Moxostoma hubbsi*, a threatened species of the same family (Catostomidae) (Branchaud and Gendron 1994). One should note that white sucker females analyzed for vitamin A in this study were all running-ripe fish and that all non-running-ripe females were released. There may have been differences between liver and egg vitamin A concentrations between running-ripe and non-running-ripe females.

Overall, the results of the present study confirm our preliminary observations concerning the elevated prevalence of malformations in white sucker caught in the Rivière des Prairies near Montreal. The study also reveals that adult suckers from the Rivière des Prairies have extremely low vitamin A stores in contrast to adults from the reference site, which appear to increase their liver retinoid stores progressively as they grow older. Whether the low retinoid stores in white suckers are related to ecological factors, in addition to contaminant factors, is not known. The results suggest that some factor intrinsic to the fertilized egg (e.g., coplanar PCBs), or a combination of intrinsic factors, is at least partially responsible for the slightly elevated prevalence of malformations among Rivière des Prairies larvae.

Acknowledgements

The research was funded by the Natural Sciences and Engineering Research Council of Canada, the Wildlife Toxicology Fund (World Wildlife Fund and Environment Canada), The Max Bell Foundation, and the Centre Saint-Laurent. Pierre Dumont of the ministère du Loisir de la Chasse et de la Pêche, Québec, arranged technical support in the field. Assistance with field work was also contributed by Dominique Mercier, Jean-Claude Deslandes, and Michel La Haye. Authentic 3,4-didehydroretinyl acetate was the generous gift of Drs. Sherry Tanumihardjo and Arun Barua, Iowa State University.

References

Barua, A.B., and U.C. Goswami. 1977. Formation of vitamin A in a freshwater fish: isolation of retinoic acid. Biochem. J. 164: 133–136.

Barua, A.B., and J.A. Olson. 1984. Synthesis of 4,4′-difluoro analogs of retinol and retinoic acid. J. Lipid Res. 25: 304–309.

Bengtsson, B-E., Å. Bengtsson, and M. Himberg. 1985. Fish deformities and pollution in some Swedish waters. Ambio, 14: 32–35.

Berra, T.M., and R.-J. Au. 1981. Incidence of teratological fishes from Cedar Fork Creek, Ohio. Ohio J. Sci. 85: 225–229.

Bitman, J., H.C. Cecil, and S.J. Harris. 1972. Biological effects of polychlorinated biphenyls in rats and quail. Environ. Health Perspect. 1: 145–149.

Blaner, W.S., and Olson, J.A. 1994. Retinol and retinoic acid metabolism. *In* The retinoids: biology, chemistry and medicine. *Edited by* M.B. Sporn, A.B. Roberts, and D.S. Goodman. Raven Press, New York. pp. 229–255.

Boily, M.H., L. Champoux, D.H. Bourbonnais, J.L. DesGranges, J. Rodrigue, and P.A. Spear. 1994. β-Carotene and retinoids in eggs of Great Blue Herons (*Ardea herodias*) in relation to St. Lawrence River contamination. Ecotoxicology. 3: 271–286.

Branchaud, A., and A. Gendron. 1994. Artificial spawning and rearing of the copper redhorse, *Moxostoma hubbsi*. Can. Field-Nat. 107: 279–282.

Chen, L.-C., I. Berberian, B. Koch, M. Mercier, V. Azais-Braesco, H.P. Glauert, C.K. Chow, and L.W. Robertson. 1992. Polychlorinated and polybrominated biphenyl congeners and retinoid levels in rat tissues: structure–activity relationships. Toxicol. Appl. Pharmacol. 114: 47–55.

Dawson, C.E. 1964. A bibliography of anomalies of fish. Gulf Res. Rep. 1: 308–399.

Dawson, C.E. 1966. A bibliography of anomalies of fish. Suppl. 1. Gulf Res. Rep. 2: 169–176.

Dawson, C.E. 1971. A bibliography of anomalies of fish. Suppl. 2. Gulf Res. Rep. 3: 215–239.

Dawson, C.E., and E. Heal. 1976. A bibliography of anomalies of fish. Suppl. 3. Gulf Res. Rep. 5: 35–41.

Frolik, C.A. 1984. Metabolism of the retinoids. *In* The retinoids. Vol. 2. *Edited by* M.B. Sporn, A.B. Robert, and D.S. Goodman. Academic Press, Montréal. pp. 177–208.

Furr, H.C. 1990. Reversed-phase high-performance liquid chromatography of retinyl esters. Methods Enzymol. 189: 85–94.

Ganguly, J. 1989. Biochemistry of vitamin A. CRC Press, Boca Raton, Fla.

Gilbert, N.L., M.-J. Cloutier, and P.A. Spear. 1995. Retinoic acid hydroxylation in rainbow trout (*Onchorynchus mykiss*) and the effect of a coplanar PCB, 3,3′4,4′-tetrachlorobiphenyl. Aquat. Toxicol. 32: 177–187.

Gilbertson, M., T. Kubiak, J. Ludwig, and G. Fox. 1991. Great lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. J. Toxicol. Environ. Health, 33: 455–520.

Gillespie, R.B., and P.C. Baumann. 1986. Effects of high tissue concentrations of selenium on reproduction by bluegills. Trans. Am. Fish. Soc. 115: 208–213.

Goswami, U.C., and A.B. Barua. 1981. Distribution of retinol and dehydroretinol in freshwater fish. Indian J. Biochem. Biophys. 18: 383–385.

Goulet, M., and D. Laliberté. 1982. BPC: contamination du milieu aquatique au Québec méridional. Publication Q.E.-53, Envirodoq ND-83-0018. Service de la qualité des eaux, ministère de l'Environnement, Québec.

Hansen, P.-D., H. von Westernhagen, and H. Rosenthal. 1985. Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. Mar. Environ. Res. 15: 59–76.

Helder, T. 1980. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on early life stages of the pike (*Esox lucius* L.). Sci. Total Environ. 14: 255–264.

Hodson, P.V., P.J. Kloepper-Sams, K.R. Munkittrick, W.L. Lockhart, D.A. Metner, P.L. Luxon, I.R. Smith, M.M. Gagnon, M. Servos, and J.F. Payne. 1991. Protocols for measuring mixed function oxygenases of fish liver. Can. Tech. Rep. Fish. Aquat. Sci. No. 1829.

Hoffman, D.J., B.A. Rattner, L. Sileo, D. Docherty, and T.J. Kubiak. 1987. Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. Environ. Res. 42: 176–184.

Hogan, J.W., and J.L. Brauhn. 1975. Abnormal rainbow trout fry from eggs containing high residues of a PCB (Aroclor 1242). Prog. Fish-Cult. 37: 229–230.

Karås, P., E. Neuman, and O. Sandström. 1991. Effects of a pulp mill effluent on the population dynamics of perch, *Perca fluviatilis*. Can. J. Fish. Aquat. Sci. 48: 28–34.

Lindesjöö, E., and J. Thulin. 1992. A skeletal deformity of northern pike (*Esox lucius*) related to pulp mill effluents. Can. J. Fish. Aquat. Sci. 49: 166–172.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

Mac, M.J., and C.C. Edsall. 1991. Environmental contaminants and reproductive success of lake trout in the Great Lakes: an epidemiological approach. J. Toxicol. Environ. Health, 33: 375–394.

Mac, M.J., C.C. Edsall, and J.G. Seelye. 1985. Survival of lake trout eggs and fry reared in water from the upper Great Lakes. J. Gt. Lakes Res. 11: 520–529.

Mauck, W.L., P.M. Merle, and F.L. Mayer. 1978. Effects of the polychlorinated biphenyl Aroclor 1254 on growth, survival, and bone development in brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 35: 1084–1088.

McFarlane, G.A., and W.G. Franzin. 1978. Elevated heavy metals: a stress on a population of white suckers, *Catostomus commersoni*, in Hamell Lake, Saskatchewan. J. Fish. Res. Board Can. 35: 963–970.

McMaster, M.E., G.J. Van Der Kraak, C.B. Portt, K.R. Munkittrick, P.K. Sibley, I.R. Smith, and D.G. Dixon. 1991. Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent. Aquat. Toxicol. 21: 199–218.

Mikulin, A.Y., and S.G. Soin. 1975. The functional significance of carotenoids in the embryonic development of teleosts. J. Ichthyol. (Engl. Transl.), 15: 749–759.

Munkittrick, K.R., and D.G. Dixon. 1988. Growth, fecondity, and energy stores of white sucker (*Catostomus commersoni*) from lakes containing elevated levels of copper and zinc. Can. J. Fish. Aquat. Sci. 45: 1355–1365.

Munkittrick, K.R., and D.G. Dixon. 1989. Use of white sucker (*Catostomus commersoni*) populations to assess the health of aquatic ecosystems exposed to low-level contaminant stress. Can. J. Fish. Aquat. Sci. 46: 1455–1462.

Niimi, A.J. 1983. Biological and toxicological effects of environmental contaminants in fish and their eggs. Can. J. Fish. Aquat. Sci. 40: 306–312.

Olson, J.A. 1979. A simple dual assay for vitamin A and carotenoids in human liver. Nutr. Rep. Int. 19: 807–813.

Palace, V.P., and S.B. Brown. 1994. HPLC determination of tocopherol, retinol, dehydroretinol and retinyl palmitate in tissues of lake char (*Salvelinus namaycush*) exposed to coplanar 3,3′,4,4′,5-pentachlorobiphenyl. Environ. Toxicol. Chem. 13: 473–476.

Paul, M., and D. Laliberté. 1988. Teneurs en mercure, plomb, cadmium, BPC et pesticides organochlorés des sédiments et de la chair de poissons du Fleuve St-Laurent et de la Rivière des Outaouais en 1985. Rep. No. QEN/QE-86-07. Direction de la qualité du milieu aquatique, ministère de l'Environnement, Québec.

Paul, M., and D. Laliberté. 1989. Teneurs en BPC, HAP et pesticides organochlorés dans les sédiments et les poissons des rivières L'Assomption, Richelieu, Yamaska, Saint-François et du lac Saint-Pierre en 1986. Rep. No. QEN/QE-89-02. Direction de la qualité du milieu aquatique, ministère de l'environnement, Québec.

Phillips, W.E.J. 1963. DDT and the metabolism of vitamin A and carotene in the rat. Can. J. Biochem. Physiol. 41: 1793–1802.

Poston, H.A., R.C. Riis, G.L. Rumsey, and H.G. Ketola. 1977. The effect of supplemental dietary amino acids, minerals and vitamins on salmonids fed cataractogenic diets. Cornell Vet. 67: 472–509.

Prough, R.A., M.D. Burke, and R.T. Mayer. 1978. Direct fluorometric methods for measuring mixed-function oxidase activity. Methods Enzymol. 52: 372–377.

Roberts, A.B., L.C. Lamb, and M.B. Sporn. 1980. Metabolism of all-*trans*-retinoic acid in hamster liver microsomes: oxidation of 4-hydroxy- to 4-keto-retinoic acid. Arch. Biochem. Biophys. 199: 374–383.

Rosenthal, H., and D.F. Alderdice. 1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. J. Fish. Res. Board Can. 33: 2047–2065.

Schiedt, K., F.J. Leuenberger, M. Vecchi, and E. Glinz. 1985. Absorption, retention and metabolic transformations of carotenoids in rainbow trout, salmon and chicken. Pure Appl. Chem. 57: 685–692.

Sharp, J.R. 1991. The influence of toxicants on the teratogenic response of fishes: a guide to the literature, a glossary of terms and a pictorial atlas. Trace Subst. Environ. Health, 24: 63–80.

Shih, T.W., and D.L. Hill. 1991. Conversion of retinol to retinal by rat liver microsomes. Drug Metab. Dispos. 19: 332–335.

Spear, P.A., and T.W. Moon. 1986. Thyroid – vitamin A interactions in chicks exposed to 3,4,3′,4′-tetrachlorobiphenyl: influence of low dietary vitamin A and iodine. Environ. Res. 40: 188–198.

Spear, P.A., T.W. Moon, and D.B. Peakall. 1986. Liver retinoid concentrations in natural populations of herring gulls (*Larus argentatus*) contaminated by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and in ring doves (Streptopelia risoria) injected with a dioxin analogue. Can. J. Zool. 64: 204–208.

Spear, P.A., H. Garcin, and J.-F. Narbonne. 1988. Increased retinoic acid metabolism following 3,3′,4,4′,5,5′-hexabromobiphenyl injection. Can. J. Physiol. Pharmacol. 66: 1181–1186.

Spear, P.A., D.H. Bourbonnais, D.H. Peakall, and T.W. Moon. 1989. Dove reproduction and retinoid (vitamin A) dynamics in adult females and their eggs following exposure to 3,3′,4,4′-tetrachlorobiphenyl. Can. J. Zool. 67: 908–913.

Spear, P.A., D.H. Bourbonnais, R.J. Norstrom, and T.W. Moon. 1990. Yolk retinoids (vitamin A) in eggs of the herring gull and correlations with polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Environ. Toxicol. Chem. 9: 1053–1061.

Spear, P.A., A.Y. Bilodeau, and A. Branchaud. 1992. Retinoids: from metabolism to environmental monitoring. Chemosphere, 25: 1733–1738.

Spies, R.B., and D.W. Rice. 1988. Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay. II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory. Mar. Biol. (Berlin), 98: 191–200.

Tacon, A.G.J. 1981. Speculative review of possible carotenoid function in fish. Prog. Fish-Cult. 43: 205–208.

Thaller, C., and G. Eichele. 1987. Identification and spatial distribution of retinoids in the developing chick limb bud. Nature (London), 327: 625–628.

Thompson, J.N. 1970. The role of vitamin A in reproduction. *In* The fat-soluble vitamins. *Edited by* H.F. DeLuca and J.W. Suttie. University of Wisconsin Press, Madison, Wis. pp. 267–281.

Thompson, J.N., J.M. Howell, G.A.J. Pitt, and C.I. McLaughlin. 1969. The biological activity of retinoic acid in the domestic fowl and the effects of vitamin A deficiency on the chick embryo. Br. J. Nutr. 23: 471–490.

Thunberg, T., U.G. Ahlborg, and H. Johnsson. 1979. Vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Arch. Toxicol. 42: 265–274.

Tickle, C., J. Lee, and G. Eichele. 1985. A quantitative analysis of the effect of all-*trans*-retinoic acid on the pattern of chick wing development. Dev. Biol. 109: 82–85.

Underwood, B.A. 1984. Vitamin A in animal and human nutrition. *In* The retinoids. Vol. 1. *Edited by* M.B. Sporn, A.B. Roberts, and D.S. Goodman. Academic Press, Montréal. pp. 282-392.

Van Der Kraak, G.J., K.R. Munkittrick, M.E. McMaster, C.B. Portt, and J.P. Chang. 1992. Exposure to bleached kraft pulp mill effluent disrupts the pituitary–gonadal axis of white sucker at multiple sites. Toxicol. Appl. Pharmacol. 115: 224–233.

von Westernhagen, H., H. Rosenthal, V. Dethlefsen, W. Ernst, U. Harms, and P.-D. Hansen. 1981. Bioaccumulating substances and reproductive success in baltic flounder *Platichthys flesus*. Aquat. Toxicol. 1: 85–99.

Wake, K., K. Motomatsu, H. Senoo, A. Masuda, and E. Adachi. 1986. Improved Kupffer's gold chloride method for demonstrating the stellate cells storing retinol (vitamin A) in the liver and extrahepatic organs of vertebrates. Stain Technol. 61: 193–200.

Weis, J., and P. Weis. 1977. Effects of heavy metals on the development of the killifish, *Fundulus heteroclitus*. J. Fish Biol. 11: 49–54.

Westin, D.T., C.E. Olney, and B.A. Rogers. 1985. Effects of parental and dietary organochlorines on survival and body burdens of striped bass larvae. Trans. Am. Fish. Soc. 114: 125–136.

The effects of small-scale turbulence on the ingestion rate of fathead minnow (*Pimephales promelas*) larvae

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Abstract: Small-scale turbulence increases encounter rates between planktonic predators and their prey. It has been hypothesized to lead to an increase in feeding by fish larvae, yet this has rarely been tested experimentally. We investigated the effects of turbulence on feeding by fathead minnow (*Pimephales promelas*) larvae to test the null hypothesis of no effect of turbulence on ingestion. In one experiment, larvae were exposed to four turbulent velocities from 0 to 2 $cm \cdot s^{-1}$ at a single prey concentration. Data analysis led to a rejection of the null hypothesis. Moreover these data suggested a significant nonlinear response of ingestion to increasing turbulence. In a second experiment, we varied turbulence and prey concentrations (15, 50, and 500 prey/L) simultaneously. We rejected the null hypothesis of no effect of turbulence for all prey concentrations. Results differed among prey concentrations. We observed a negative linear response for the two lower prey concentrations, but a domed response at 500 prey/L. We conclude that turbulence does influence ingestion, but not as suggested by an extension of encounter models. Thus, turbulence may not always be beneficial in the field.

Résumé : Les turbulences à petite échelle accroissent la fréquence des contacts entre les prédateurs planctoniques et leurs proies. On a avancé que ce phénomène pouvait conduire à une alimentation plus intense chez les larves des poissons, mais cette hypothèse n'avait été testée expérimentalement que rarement. Nous l'avons fait en prenant des larves du tête-de-boule (*Pimephales promelas*) et en testant l'hypothèse principale, soit d'aucun effet des turbulences sur l'ingestion. Dans un cas, des larves ont été exposées à un flot turbulent qui s'écoulait à quatre vitesses différentes, de 0 à 2 $cm \cdot s^{-1}$, et à des proies à une seule concentration. L'analyse des données nous a conduits à rejeter l'hypothèse principale. De plus, les données semblaient indiquer l'existence d'une réponse non linéaire significative de l'ingestion à la turbulence accrue. Dans un autre cas, nous avons fait varier à la fois la turbulence et la concentration des proies (15, 50 et 500 proies/L). Nous avons rejeté l'hypothèse principale d'aucun effet de la turbulence à toutes les concentrations de proies. Les résultats différaient selon la concentration des proies. Nous avons observé une relation linéaire inverse dans le cas des deux concentrations les plus faibles, mais nous avons obtenu une courbe en forme de cloche à 500 proies/L. Nous parvenons à la conclusion que les turbulences influencent réellement l'ingestion, mais pas de la manière que le laisse prévoir une extension des modèles qui décrivent les contacts prédateur–proie. Sur le terrain, ces turbulences n'ont pas toujours un effet bénéfique.

[Traduit par la Rédaction]

Introduction

Turbulence is a consistent hydrodynamic feature of all aquatic ecosystems (Thorpe 1985; Yamazaki and Osborn 1988). Typically, turbulence is confined to the surface or mixed layers of aquatic environments, although tidal currents do also produce bottom layer turbulence (Tennekes and Lumley 1972). It occurs at spatial scales small enough to affect planktonic trophodynamics via influences on phytoplankton size distributions and production of primary and

Received November 15, 1993. Accepted March 2, 1995.
J12160

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secondary producers (Oviatt 1981; Davis et al. 1991; Kiørboe 1993). For zooplankton, it has been shown to modify their behaviour (Costello et al. 1990; Haury et al. 1992; Saiz and Alcaraz 1992*a*), metabolism (Marrasé et al. 1990; Saiz and Alcaraz 1992*b*; Alcaraz et al. 1994), and growth rates (Alcaraz et al. 1988; Saiz and Alcaraz 1991; Saiz et al. 1992). Less is known of the effects of turbulence on larval fish (B.R. MacKenzie, Danish Institute for Fisheries and Marine Research, Charlottenlund Castle, Charlottenlund, DK-2920, Denmark, unpublished results).

Rothschild and Osborn (1988) have demonstrated theoretically that turbulence can enhance the rate of encounter between planktonic predators and their prey. This increased encounter rate has now been verified experimentally for zooplankton (Costello et al. 1990; Marrasé et al. 1990) and fish larvae (B.R. MacKenzie, unpublished results). The observed increases in encounter as a result of turbulence have been used to explain patterns of growth and survival of fish larvae in the field. For example, MacKenzie et al. (1990) suggested that turbulence-induced increases in feeding rates may resolve the paradox of the observation that prey concentrations in the field are often below those required to sustain fish larvae in the laboratory. Sundby and Fossum (1990) and Sundby et al. (1994) explained enhanced feeding in Arcto-Norwegian cod (*Gadus morhua*) observed during storms by invoking the beneficial effects of turbulence. Matsushita (1991) argued that the level of turbulence in the field was important in determining feeding success of Japanese anchovy (*Engraulis japonica*) and sardine (*Sardinops melanostictus*). Muelbert et al. (1994) also concluded that turbulence-related increases in feeding and growth were important factors affecting the distribution of herring larvae (*Clupea harengus*) off southwest Nova Scotia, Canada. Recently, F.E. Werner (Marine Sciences Program, University of North Carolina, College Box 3300, Chapel Hill, NC 27599-3300, U.S.A., unpublished results) has invoked turbulence to account for the growth and survival of cod and haddock (*Melanogrammus aeglefinus*) larvae on Georges Bank.

Although the link between turbulence, encounter, ingestion, growth, and survival is appealing, experimental tests of the linkages have yielded ambiguous results. Studies of copepods show evidence of beneficial effects of turbulence (Costello et al. 1990; Marrasé et al. 1990; Saiz and Alcaraz 1992*a*), but other studies have suggested detrimental effects (Oviatt 1981). For example, MacKenzie et al. (1994) predict that the effects of turbulence on feeding will be nonlinear. They predict peak levels of ingestion at intermediate levels of turbulence and lower levels of ingestion at high levels of turbulence. Furthermore, MacKenzie and Leggett (1991) and Saiz et al. (1992) suggest that the response to turbulence will be dependent on prey concentration. These authors suggest that feeding enhancement as a result of turbulence will be greatest at low prey concentrations. However, the hypothesis that the increases in encounter brought about by quantified levels of turbulence yield increases in ingestion and growth rates remains untested for fish larvae.

The objectives of our research were to determine the effects of turbulence on the ingestion rates of fish larvae. Specifically, we tested the null hypothesis that small-scale turbulence does not influence ingestion rates of larval fish. The alternative hypotheses examined were that ingestion was either linearly or nonlinearly related to the level of turbulence. In a second experiment, we investigated the interaction between turbulence and prey concentration suggested by MacKenzie and Leggett (1991) and Saiz et al. (1992).

Methods

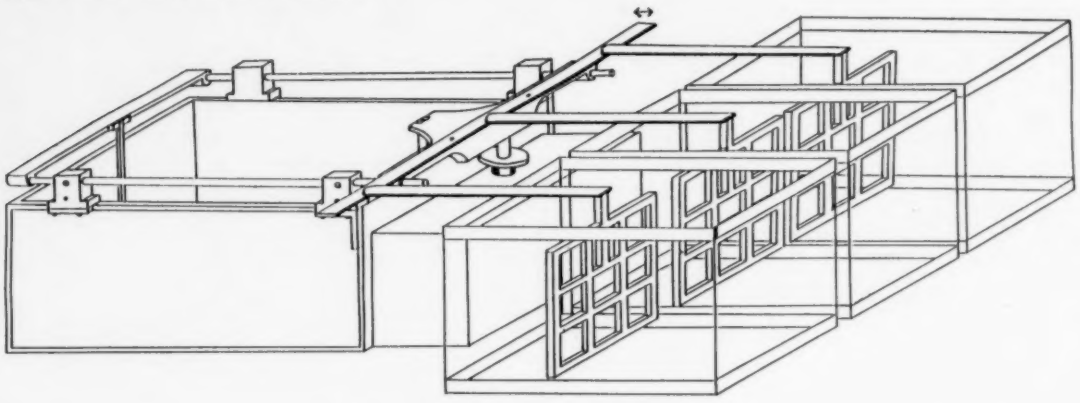
We conducted experiments using fathead minnow (*Pimephales promelas*) larvae. Fathead minnow is an intermittent, multiple spawning freshwater species that is easy to culture (Gale and Buynak 1982). Larvae hatch at approximately 5 mm total length. Five mating pairs of adults provided a continuous supply of larvae for the experiments. Each pair was reared in a 20-L aquarium at 25°C under a 16 h light : 8 h dark cycle. Spawning was induced by alterations in the temperature and light environments (Denny 1987). A polyvinyl chloride (PVC) pipe, cut in half longitudinally, served as a spawning substrate (Denny 1987). Once the eggs were laid, the substrate was removed to a brood tank, and hatching occurred after 4 days. Larvae began feeding on the day of hatching. They were fed brine shrimp (*Artemia* sp. San Francisco strain) nauplii ad libitum. This prey is within the size range of natural food for these larvae (Norberg and Mount 1985). We used 6-day-old larvae (average total length 6.5 mm) in the experiments to ensure sufficient time for conditioning to feeding, and to enable us to identify which larvae had successfully initiated feeding.

The first experiment, conducted to evaluate the effect of turbulence on feeding rate in fathead minnow larvae, involved four levels of turbulence in a gradient treatment, at a single prey concentration (30 prey/L). This concentration was chosen because Miller et al.'s (1992) data suggest this should be a nonsaturating prey concentration. The experiment was conducted in eight complete blocks. Mean number of prey per gut was the response variable. Data were analyzed as a one-way analysis of variance with turbulence and squared turbulence as covariates. We incorporated a second-order polynomial to differentiate between the competing alternative hypotheses.

Trials were conducted in 20-L aquaria (40 × 20 × 25 cm) in complete blocks. Each aquarium held ten 6-day-old larvae. Twenty hours prior to a trial, larvae were transferred to each experimental tank by gently spooning them from stock tanks. This period ensured full gut clearance prior to the trial. Each trial allowed the larvae to feed for a period of 15 min while being exposed to a level of turbulence. At the end of the trial, the larvae were immediately removed from each tank using a small dip net and killed in a chilled solution of MS-222 prior to final preservation in a 70% ethanol solution. A visual check was made to ensure no regurgitation occurred prior to preservation. Subsequently, larvae were individually dissected under a dissecting microscope to count the *Artemia* sp. in their stomachs.

To obtain the prey concentration required in the tanks for each trial, we added a prescribed volume of a known concentration of nauplii. The actual prey concentration in each tank was sampled during each trial by lowering three PVC

Fig. 1. Scale diagram of the oscillating grid apparatus. The grids were made out of 1-cm square stock Plexiglas. The grid was 19 × 19 cm and had a mesh opening of 5 cm. The oscillating motion was generated by an American Optical (model 02156) shaking water bath. The stroke of the oscillation was 2.8 cm. The arrow indicating the oscillation is drawn to scale. The distance from the midpoint of the grid's stroke to the centre of the tank was 3.6 cm. Three 20-L aquaria were filled with 17 L of water.



tubes (4 cm diameter) over stoppers placed randomly on the bottom of each tank. These samples were taken 3 min after the prey were added. The volume of each sample was measured (~175 mL) and the sample was then poured through a 64-μm mesh sieve to retain the *Artemia* sp. for later enumeration.

Turbulence was generated by an oscillating grid. The grid design was based upon one used in two previous studies (Thompson and Turner 1975; Hopfinger and Toly 1976). We used shaking water baths to oscillate a Plexiglas mesh grid (Fig. 1). Each water bath generated turbulence in three experimental chambers. Owing to both the size of the chamber and the apparatus used, turbulence was not uniform throughout the tank. We characterized the level of turbulence in the tank as the turbulent velocity (u, in $cm \cdot s^{-1}$) present in the centre of the tank by applying Hopfinger and Toly's (1976) equation to our system, so that

$$[1] \quad u = C \cdot f \cdot S^{1.5} \cdot M^{0.5} \cdot z^{-1}$$

where C is a constant of proportionality equal to 2.5×10^{-1}, f is the frequency of the oscillating grid, S is the stroke (2.8 cm), M is the mesh size (5.0 cm), and z is the distance from the midpoint of the grid's stroke to the center of the tank (3.6 cm). The stroke used in the trials, 2.8 cm, reduced the probability of the larvae hitting the grid. We used four different frequencies of oscillation (0, 0.9, 1.8, and 2.7 Hz), which generated calculated turbulent velocities of 0, 0.65, 1.31, and 1.96 $cm \cdot s^{-1}$ in the middle of the tank. Turbulence is usually reported in the literature as the energy dissipation rate (ϵ) in $cm^2 \cdot s^{-3}$. A typical value of ϵ, reported to be beneficial to ingestion in fish larvae, is 10^{-3} $cm^2 \cdot s^{-3}$ (Marrasé et al. 1990). Using this value in Rothschild and Osborn's (1988) turbulent velocity equation (their eq. 4), and an estimated larval reactive distance of 0.65 cm, the approximate equivalent turbulent velocity is 0.2 $cm \cdot s^{-1}$.

The second experiment investigated the effects of turbulence and prey concentration simultaneously. The experiment employed a randomized complete block design replicated eight times. We used the same four levels of turbulence in a gradient treatment design as in the first experiment. Prey concentration (15, 50, and 500 prey/L) was considered a factorial treatment. We hypothesized that we would see no influence of turbulence if we exposed larvae to a prey concentration that permitted them to feed at a maximal rate and thus we chose three prey concentrations to ensure that we would bracket both a nonsaturating and a saturating prey concentration. We chose 15 and 50 prey/L to ensure that the prey concentration differences would be sufficiently large that we could maintain them during trials. Thus, 15 prey/L may have been anywhere from 10 to 20 but never overlapped with the 50 prey/L concentration. The prey concentrations we employed were typical of those found in the field (Pace 1984, 1986; Wu and Culver 1991). Experimental procedures were the same as in the first experiment. The data were analyzed as a two-way analysis of variance with turbulence and squared turbulence as covariates.

Results

There were few subjective differences between the behaviour of larvae in the nonturbulent and turbulent aquaria. Larval swimming speeds and patterns appeared similar in the two cases. Larvae did avoid the area of the tank directly in the path of the grid in turbulent trials. However, because the area of the tank that was directly affected by the motion of the grid was small, this did not appear to restrict the overall movements of larvae in the turbulent environment.

The first experiment was designed to test the null hypothesis of no effect of turbulence on ingestion. The average number of prey eaten was affected by turbulence (Fig. 2). Overall the model was significant ($F_{[9,22]} = 21.56$, $p < 0.001$) with $R^2 = 0.90$ (Table 1). Therefore, we rejected our null hypothesis and concluded that turbulence did influence ingestion rate. Furthermore, the parameter estimate for squared turbulence was also significant ($turb^2$, $F_{[1,22]} =$

Fig. 2. Relationship between feeding rate of fathead minnow larvae and turbulence. Data are given as means ± SE from eight replicates in which each replicate contained 10 larvae for each level of turbulence. The larvae were exposed for 15 min to a single concentration (30 prey/L) of *Artemia* sp. prey. Turbulence values are given as grid frequency (Hz) and turbulent velocity ($cm \cdot s^{-1}$), which are estimated values for the center of the tank (see eq. 1).

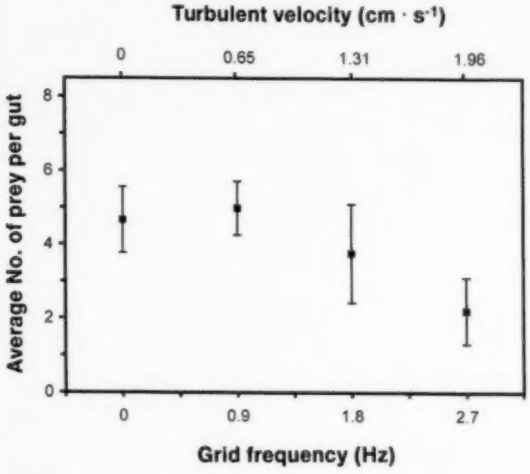
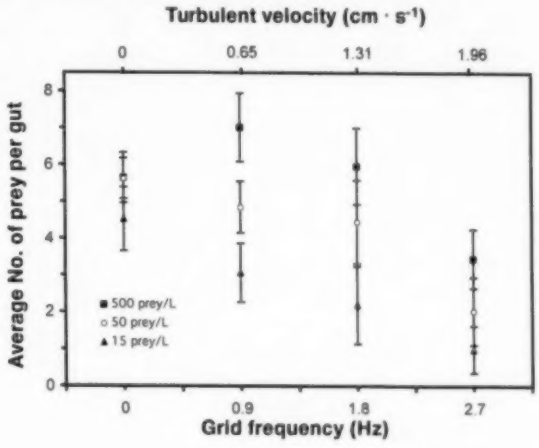


Fig. 3. Relationship between mean gut content per tank with turbulence as a function of prey concentration. Data are given as means ± SE of eight replicates in which each replicate contained 10 larvae for each level of turbulence. The larvae were exposed for 15 min to a single concentration of *Artemia* sp. prey. The prey concentrations were 15, 50, and 500 prey/L. Turbulence values are estimates for the centre of the tank (see eq. 1).



5.91, $p < 0.03$). Moreover, the quadratic term was negative, indicative of a nonlinear response of feeding rate to increasing turbulence. Single degree of freedom contrasts suggested a nonsignificant enhancement of feeding at low turbulence (0.9 Hz), and a significant reduction in feeding at the highest level (2.7 Hz) employed (Fig. 2).

The results from the second experiment yielded significant differences between prey concentrations (Fig. 3). Exposure to the highest level of turbulence led to a decline in ingestion at all prey concentrations, as in the first experiment. This led to a rejection of the null hypothesis of no effect of turbulence on ingestion. However, in contrast to the first experiment, enhanced feeding at low levels of turbulence was only observed at the high prey concentration. At 500 prey/L we documented a 1.24-fold increase in feeding from zero turbulence to the next level (0.9 Hz). However, at 15 and 50 prey/L, feeding rates declined as turbulence increased without any statistically significant evidence of nonlinearity as seen in the earlier experiment. Thus, we could not distinguish between the competing alternative hypotheses. Analysis indicated that both prey concentration and turbulence had significant effects (prey: $F_{[2,84]} = 10.87$, $p < 0.001$; turbulence: $F_{[3,84]} = 8.01$, $p < 0.001$; Table 2) on the feeding rate of the fathead minnow larvae. There was no significant interaction effect ($F_{[6,84]} = 0.65$, $p < 0.69$; Table 2). Thus, while prey concentration was clearly a factor that affected the relationship between ingestion and turbulence, we could not determine the nature of its effects from these data. There was a significant negative linear relationship for the lowest prey concentration and a significant nonlinear relationship for the highest prey concentration (Table 2). However, within the 50 prey/L trial, because of the larger variability in the results we were unable to distinguish between the null hypothesis and the alternatives.

Table 1. Parameter estimates for the effects of turbulence on feeding rate at a prey concentration of 30 prey/L.

	Intercept	Linear	Quadratic	R^2
t statistic	5.12**	0.91	−2.43*	0.90
Parameter estimate	3.243	0.610	−0.579	
SE	0.63	0.67	0.24	

Note: The experiment was conducted in eight complete blocks involving four levels of turbulence (0, 0.9, 1.8, and 2.7 Hz). Each block used four tanks, each containing 10 larvae. Analyses were conducted on mean gut contents per tank using an analysis of variance (ANOVA). The overall ANOVA was significant ($F_{[9,22]} = 21.56$, $p < 0.001$). Tests of significance for the overall regression are based upon 32 observations, and individual parameter significance levels are based on a *t* test with 1 degree of freedom.

*Significant at $0.01 < p < 0.05$.

**Significant at $p < 0.01$.

Discussion

Our data suggest that the effects of small-scale turbulence on feeding rate in fish larvae are complex. For the four prey concentrations we tested, we were able to reject our null hypothesis of no effect of turbulence on ingestion in three instances, yet we could not distinguish clearly between the alternative hypotheses of linear and nonlinear effects. However, our findings indicate that while encounter rates may

Table 2. Parameter estimates for the effect of turbulence on feeding rate at a three prey concentrations.

	Intercept	Linear	Quadratic	R^2
15 prey/L				
t statistic	8.07**	−2.10*	0.32	0.86
Parameter estimate	5.439	−1.495	0.081	
SE	0.67	0.71	0.25	
50 prey/L				
t statistic	6.60**	0.14	−1.51	0.76
Parameter estimate	5.943	0.131	−0.509	
SE	0.90	0.95	0.34	
500 prey/L				
t statistic	8.66**	3.29**	−4.65**	0.86
Parameter estimate	5.929	2.379	−1.196	
SE	0.68	0.72	0.26	

Note: The experiment was a 4 by 3 factorial design replicated eight times. Overall, both turbulence ($F_{[3,84]} = 8.01$, $p < 0.001$) and prey concentration ($F_{[2,84]} = 10.87$, $p < 0.001$) were significant. Analyses were then conducted treating turbulence as a gradient treatment design. Analyses were conducted on mean gut contents per tank. R^2 values for each regression are based upon 32 observations, and individual parameter significance levels are based on a *t* test with 1 degree of freedom.

*Significant at $0.01 < p < 0.05$.

**Significant at $p < 0.01$.

increase in turbulent environments, the extension to assuming that ingestion will similarly increase is unwarranted. In all cases, we document a decrease in ingestion at the highest level of turbulence. Furthermore, in two instances, we show evidence for a nonlinear response in ingestion. However, as the instances of nonlinearity we report were not consistent among food concentrations, caution must be exercised in interpreting these results. We suggest that the potential nonlinear response of ingestion rate by larval fish to increases in turbulence requires further study.

Despite the conflicting data with regard to the exact form of the relationship, our results clearly demonstrate negative effects on ingestion at high levels of turbulence. MacKenzie and Leggett (1991) suggested that small-scale turbulence associated with frontal and upwelling regions may increase larval ingestion rates. The assumption made in deriving this hypothesis was that turbulence-induced increases in encounter would translate into increases in ingestion. Our results suggest that if increases in ingestion in such regions are observed, they may more reasonably reflect hydrodynamically induced increases in prey abundance than turbulence-induced increases in ingestion. However, MacKenzie and Leggett's hypothesis may still hold if the nonlinear response that occurred in two instances in our study is typical. Under these conditions, frontal and upwelling regions may well provide the double benefit of increased prey abundance and increased ingestion probability.

The negative effect of turbulence observed in this experiment can be explained by several mechanisms. Generally prey are patchily distributed. Turbulence may act to disrupt local food concentrations; this would lead to a perceived reduction in prey concentration and thus reduced ingestion rates (Peterman and Bradford 1987; Wroblewski and Richman 1987). The impact of turbulence may be related to larval reaction times. If larvae are too slow to respond to prey whose relative velocities are increased by small-scale turbulence, ingestion rates may decrease (Drost et al. 1988; Matsushita 1991). Finally, as suggested by MacKenzie et al. (1994), the energy in the turbulent eddies may be too high, such that even if the fish responds, its rate of successful capture and hence ingestion rate will decrease.

Other studies provide support for the nonlinear relationship between feeding rate and turbulence suggested in two instances by our data. Recently, MacKenzie et al. (1994) predicted, theoretically, a nonlinear response of ingestion to increasing turbulence for fish larvae. They suggested that this response occurs because, while encounter increases with turbulence, the probability of successful pursuit decreases as turbulence increases. In a similar theoretical development Matsushita (1991) also predicted a nonlinear response of ingestion to turbulence for fish larvae. Empirical evidence from copepods also suggests a nonlinear response. Saiz et al. (1992) reported an enhancement of feeding for *Acartia* sp. at low intensities of turbulence and a decrease in feeding at higher intensities of turbulence. The authors suggested that higher intensities of turbulence caused a detrimental effect on the feeding currents created by the copepod's appendages. Similarly Marrasé et al. (1990) showed a dome-shaped response of ingestion to turbulence in *Centropages hamatus*. In this case, the energetic cost of feeding in a turbulent regime was suggested as the mechanism causing the nonlinearity. Less direct empirical evidence for a nonlinear response to turbulence comes from studies on recruitment patterns in fishes. Cury and Roy (1989) reported that high levels of turbulence induced by upwelling were detrimental to recruitment of North African pelagic marine fishes, and that recruitment was highest at intermediate levels of wind stress. Subsequently, Ware and Thomson (1991) found similar patterns in recruitment time-series data for Pacific sardine (*Sardinops sagax*).

In summary, we have documented negative effects of turbulence on feeding rates in larval fish in a series of laboratory experiments. Our results suggest that the effect of turbulence may be nonlinear at certain prey concentrations. Although it is difficult to transfer these results directly to the field situation, our findings do suggest that the assumption that because turbulence increases encounter rates feeding rates will also increase may be inaccurate. We suggest that the influence of turbulence on feeding, growth, and distribution of fish is complicated, largely unknown, and warrants further study.

Acknowledgements

We thank T. Herra for assistance in the laboratory and T. Bernhardt for drafting Fig. 1. Pierre Pépin and Thomas Kiørboe provided critical comments on an earlier version of the manuscript. Funding was provided by OPEN (the Ocean Production Enhancement Network), one of 15 Networks of Centres of Excellence supported by the Government of Canada, through the Natural Sciences and Engineering Council of Canada, and by a grant from Fonds pour la formation de chercheurs et l'aide à la recherche (FCAR) to W.C.L.

References

Alcaraz, M., E. Saiz, C. Marrasé, and D. Vaqué. 1988. Effects of turbulence on the development of phytoplankton biomass and copepod populations in marine microcosms. Mar. Ecol. Prog. Ser. 49: 117–125.

Alcaraz, M., E. Saiz, and A. Calbert. 1994. Small-scale turbulence and zooplankton metabolism: effects of turbulence on heartbeat rates and planktonic crustaceans. Limnol. Oceanogr. 39: 1465–1470.

Costello, J.H., J.R. Strickler, C. Marrasé, G. Trager, R. Zeller, and A.J. Freise. 1990. Grazing in a turbulent environment: behavioural response of a calanoid copepod, *Centropages hamatus*. Proc. Natl. Acad. Sci. U.S.A. 87: 1648–1652.

Cury, P., and C. Roy. 1989. Optimal environmental window and pelagic fish recruitment success in upwelling areas. Can. J. Fish. Aquat. Sci. 46: 670–680.

Davis, C.S., G.R. Flierl, P.H. Wiebe, and P.J.S. Franks. 1991. Micropatchiness, turbulence and recruitment in plankton. J. Mar. Res. 49: 109–151.

Denny, J. 1987. Guidelines for the culture of fathead minnows (*Pimephales promelas*) for use in toxicity tests. U.S. Environ. Prot. Agency Res. Dev. Rep. No. EPA/600/3-87/001.

Drost, M.R., J.W.M. Osse, and M. Muller. 1988. Prey capture by fish larvae, water flow patterns and the effects of escape movements of prey. Neth. J. Zool. 38: 23–45.

Gale, W.F., and G.L. Buynak. 1982. Fecundity and spawning frequency of the fathead minnow: fractional spawner. Trans. Am. Fish. Soc. 111: 35–40.

Haury, L.R., H. Yamazaki, and C.L. Fey. 1992. Simultaneous measurements of small-scale physical dynamics and zooplankton distributions. J. Plankton Res. 14: 513–530.

Hopfinger, E.J., and J.-A. Toly. 1976. Spatially decaying turbulence and its relation to mixing across density interfaces. J. Fluid Mech. 78: 155–175.

Kiørboe, T. 1993. Turbulence, phytoplankton cell size, and the structure of pelagic food webs. Adv. Mar. Biol. 29: 1–72.

MacKenzie, B.R., and W.C. Leggett. 1991. Quantifying the contribution of small-scale turbulence to the encounter rates between larval fish and their zooplankton prey: effects of wind and tide. Mar. Ecol. Prog. Ser. 73: 149–160.

MacKenzie, B.R., W.C. Leggett, and R.H. Peters. 1990. Estimating larval fish ingestion rates: can laboratory derived values be reliably extrapolated to the wild? Mar. Ecol. Prog. Ser. 67: 209–225.

MacKenzie, B.R., T.J. Miller, S. Cyr, and W.C. Leggett. 1994. Evidence for a dome-shaped relationship between turbulence and larval fish ingestion rates. Limnol. Oceanogr. 39: 1790–1799.

Marrasé, C., J.H. Costello, T. Granata, and J.R. Strickler. 1990. Grazing in a turbulent environment: energy dissipation, encounter rates, and efficacy of feeding currents in *Centropages hamatus*. Proc. Natl. Acad. Sci. U.S.A. 87: 1653–1657.

Matsushita, K. 1991. How do larvae of limited motility encounter nauplii in the sea? Bull. Plankton Soc. Jpn. (Spec. Conf.), 1991: 251–270.

Miller, T.J., L.B. Crowder, J.A. Rice, and F.P. Binkowski. 1992. Body size and the ontogeny of the functional response in fishes. Can. J. Fish. Aquat. Sci. 49: 805–812.

Muelbert, J.H., M.R. Lewis, and D.E. Kelley. 1994. The importance of small-scale turbulence in the feeding of herring larvae. J. Plankton Res. 16: 927–944.

Norberg, T.J., and D.I. Mount. 1985. A new subchronic fathead minnow (*Pimephales promelas*) toxicity test. Environ. Toxicol. Chem. 4: 711–718.

Oviatt, C.A. 1981. Effects of different mixing schedules on phytoplankton, zooplankton, and nutrients in marine microcosms. Mar. Ecol. Prog. Ser. 4: 57–67.

Pace, M.L. 1984. Zooplankton community structure, but not biomass, influences the phosphorous – chlorophyll *a* relationship. Can. J. Fish. Aquat. Sci. 41: 1089–1096.

Pace, M.L. 1986. An empirical analysis of zooplankton community size structure across lake trophic gradients. Limnol. Oceanogr. 31: 45–55.

Peterman, R.M., and M.J. Bradford. 1987. Wind speed and mortality rate of a marine fish, the northern anchovy (*Engraulis mordax*). Science (Washington, D.C.), 235: 354–356.

Rothschild, B.J., and T.R. Osborn. 1988. Small-scale turbulence and plankton contact rates. J. Plankton Res. 10: 465–474.

Saiz, E., and M. Alcaraz. 1991. Effects of small-scale turbulence on development time and growth of *Acartia grani* (Copepoda: Calanoida). J. Plankton Res. 13: 873–883.

Saiz, E., and M. Alcaraz. 1992*a*. Free-swimming behaviour of *Acartia clausi* (Copepoda: Calanoida) under turbulent water movement. Mar. Ecol. Prog. Ser. 80: 229–236.

Saiz, E., and M. Alcaraz. 1992*b*. Enhanced excretion rates induced by small-scale turbulence in *Acartia* (Copepoda: Calanoida). J. Plankton Res. 14: 681–689

Saiz, E., M. Alcaraz, and G.-A. Paffenhofer. 1992. Effects of small-scale turbulence on feeding rate and gross-growth efficiency of three *Acartia* species (Copepoda: Calanoida). J. Plankton Res. 14: 1085–1097.

Sundby, S., and P. Fossum. 1990. Feeding conditions of Arcto-Norwegian cod larvae compared with Rothschild and Osborn theory on small-scale turbulence and plankton contact rates. J. Plankton Res. 12: 1153–1162.

Sundby, S., B. Ellertsen, and P. Fossum. 1994. Encounter rates between first-feeding cod larvae and their prey during moderate to strong turbulent mixing. ICES Mar. Sci. Symp. 198: 393–405.

Tennekes, H., and J.L. Lumley. 1972. A first course in turbulence. MIT Press, Cambridge, Mass.

Thompson, S.M., and J.S. Turner. 1975. Mixing across an interface due to turbulence generated by an oscillating grid. J. Fluid Mech. 67: 349–368.

Thorpe, S.A. 1985. Small-scale processes in the upper ocean boundary layer. Nature (London), 318: 519–522.

Ware, D.M., and R.E. Thomson. 1991. Link between log-term variability in upwelling and fish production in the northeast Pacific Ocean. Can. J. Fish. Aquat. Sci. 48: 2296–2306.

Wroblewski, J.S., and J.G. Richman. 1987. The non-linear response of plankton to wind mixing events: implications for larval survival of northern anchovy. J. Plankton Res. 9: 103–123.

Wu, L., and D.A. Culver. 1991. Age-0 yellow perch (*Perca flavescens*) dietary ontogeny in the western basin of Lake Erie: effect of midsummer decline in zooplankton populations. Verh. Int. Ver. Theor. Angew. Limnol. 24: 2366–2370.

Yamazaki, H., and T.R. Osborn. 1988. Review of oceanic turbulence: implications for biodynamics. *In* Toward a theory on biological–physical interactions in the world ocean. *Edited by* B.J. Rothschild. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 215–234.

Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) populations in the Atlantic Ocean

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Abstract: Pacific, Atlantic, and Mediterranean populations of the swordfish (*Xiphias gladius*) are currently considered as separate fisheries management units, and populations in different regions of the Atlantic are thought to constitute different stocks on the basis of recapture data. To test these hypotheses we sequenced hypervariable segments of the control region of mitochondrial DNA in 35 swordfish from three regions of the Atlantic, as well as in 8 and 7 individuals from the Pacific and Mediterranean regions, respectively. Sixty of the 81 variable sites were confined to a 280 base pair stretch in the left domain of the control region, indicating that this segment is a rich source of genetic markers. Thirty-three haplotypes were found that could be assigned to two clades differing by 3.8% on average, and that diverged approximately 550 000 years ago. Clade I haplotypes were ubiquitous, but haplotypes from clade II predominated in the Mediterranean, and thus likely originated there during Pleistocene marine regressions. Overall, we conclude that there is extensive mixing of swordfish within the Atlantic Ocean. Sister-group relationships of haplotypes from different oceans indicate historical gene flow between these populations, but the co-occurrence of the same haplotypes in different oceans must reflect recent or ongoing dispersal.

Résumé : En gestion des pêches, les populations d'espadons (*Xiphias gladius*) du Pacifique, de l'Atlantique et de la Méditerranée sont actuellement considérées comme des unités distinctes; par ailleurs, d'après les données de recapture, les populations des différentes régions de l'Atlantique constitueraient des stocks distincts. Pour vérifier ces hypothèses, nous avons séquencé des segments hypervariables de la région de contrôle de l'ADN mitochondrial de 35 espadons provenant de trois régions de l'Atlantique et de 8 et 7 sujets provenant des régions du Pacifique et de la Méditerranée, respectivement. Sur les 81 sites de variabilité étudiés, 60 sont confinés à un segment de 280 paires de bases situé dans la partie gauche de la région de contrôle, ce qui indique que ce segment est riche en marqueurs génétiques. Nous avons trouvé 33 haplotypes attribuables à deux clades différant en moyenne de 3,8%; ces deux clades se sont séparés il y a environ 550 000 ans. Les haplotypes du clade I étaient présents partout; quant à deux du clade II, ils prédominaient dans la Méditerranée, ce qui signifie qu'ils sont probablement apparus dans cette région, durant les régressions marines du Pléistocène. Nous sommes arrivés à la conclusion qu'il y a un mélange considérable chez l'espadon de l'océan Atlantique. Les relations caractéristiques des groupes-frère que nous avons constatées entre les groupes d'haplotypes de divers océans indiquent qu'il y a eu par le passé un flux génétique entre les populations, mais la présence de ces mêmes haplotypes dans différents océans est probablement due à une dispersion récente, sinon actuelle.

[Traduit par la Rédaction]

Introduction

The degree of geographic structuring of mitochondrial DNA (mtDNA) in highly mobile vertebrate species reflects both present and historical levels of gene flow among populations (Avise et al. 1992; Quinn 1992; Wenink et al. 1993). Gene flow can act as a strong homogenizing force by reducing genetic differentiation among populations

Received October 17, 1994. Accepted March 7, 1995.
J12605

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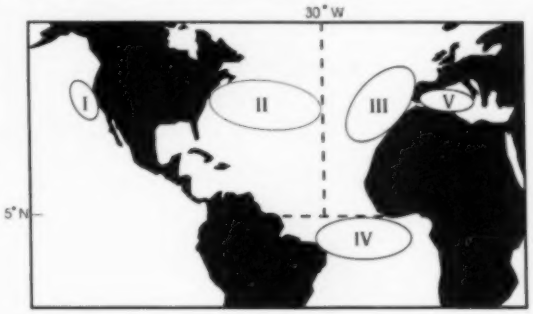
(Slatkin 1987), and together with the stochastic extinction of mtDNA variants it will eventually reduce the diversity of haplotypes in populations (Avise et al. 1987). Many vertebrate species with life histories conducive to dispersal conform to this phylogeographic pattern, where a sample of individuals collected over a vast geographical area exhibit just a few mtDNA variants, and low levels of genetic diversity (e.g., Graves et al. 1984; Ball et al. 1988). However, some highly migratory species possess divergent mtDNA lineages that are codistributed throughout their range. For example, geographic co-occurrence of divergent haplotypes has been described in the lesser snow goose (*Chen caerulescens caerulescens*), a species renowned for the strong philopatry of females to their breeding grounds (Cooke et al. 1975). Fixed differences between these mtDNA lineages are thought to have been established in allopatry, and thus the present distribution of haplotypes reflects secondary expansion and mixing of the lineages (Avise et al. 1992; Quinn 1992).

High dispersal capabilities and the absence of physical barriers to gene flow do not necessarily translate into panmixia. Populations can be effectively subdivided by strong maternal philopatry to breeding grounds, resulting in the establishment of fixed differences between them as mutations accumulate over time. Close phylogeographic association of maternal lineages with breeding areas is exemplified by the Canada goose (*Branta canadensis*) (Van Wagner and Baker 1990), the dunlin (*Calidris alpina*) (Wenink et al. 1993), and the green turtle (*Chelonia mydas*) (Bowen et al. 1992).

Despite their strong dispersal capabilities, swordfish (*Xiphias gladius*) sampled in the North Atlantic Ocean have divergent mtDNA haplotypes in the absence of geographic isolation (Alvarado Bremer 1992; J.R. Alvarado Bremer, unpublished results), and the origin of these variants needs to be investigated. This cosmopolitan monotypic species spawns year round in most circumtropical waters deeper than 200 m. Although there are sampling biases in the data (certain seasons and areas of the Atlantic Ocean being selected over others), it does appear as if spawning peaks in the winter (Grall et al. 1983). This view is corroborated by recent analysis of gonadal maturity of swordfish, which places the major breeding grounds for the Northwest Atlantic between 18 and 35°N, and west of 60°W (Arocha and Lee 1993). Spawning data outside the North Atlantic are even more scarce, but there are at least two spawning areas in the Mediterranean Sea (Cavaliere 1963; de la Serna et al. 1992), and some distinct spawning areas have been suggested for the Pacific Ocean (Matsumoto and Kazama 1974). If these spawning grounds are truly separated, some level of phylogeographic structuring in mtDNA might be expected.

For commercial fisheries purposes, the Pacific, Atlantic, and Mediterranean populations are considered separate management units that have very little or no intermixing. In addition, several scenarios of population stock subdivision have been proposed for the Atlantic Ocean (see Fig. 1), mostly on the basis of evidence from tagging programs (International Commission for the Conservation of Atlantic Tunas 1993). Recaptures of tagged swordfish have revealed that although they are capable of journeys of several thousand

Fig. 1. Sampling regions used in this study and proposed population stock structure of the swordfish in the Atlantic Ocean. The Mediterranean Sea and the Pacific Ocean are considered to be separate units. Regions are as follows: I, Pacific; II, Northwest Atlantic; III, Northeast Atlantic; IV, South Atlantic; V, Mediterranean Sea.



kilometres (International Commission for the Conservation of Atlantic Tunas 1993), females in the Northwest Atlantic display strong homing abilities towards feeding areas. Most recaptures of large females by the Canadian Large Pelagic Fish Tagging Program, for example, occurred within a few kilometres of the release site many years later (Burnett et al. 1987). Additionally, females segregate from males during the summer, and regularly move from tropical waters in the Caribbean and Gulf of Mexico to the cooler waters off the United States and Canada. The most important evidence suggesting that there are separate eastern and western stocks of the swordfish in the Atlantic is that no transoceanic recaptures have been recorded. This has been considered by some fisheries biologists as sufficient justification to separate the North Atlantic into western and eastern stocks at 30°W. There are also no records of recaptures between the northern and southern hemispheres, and so an arbitrary line at latitude 5°N divides the Atlantic into northern and southern stocks (International Commission for the Conservation of Atlantic Tunas 1993). Unfortunately, the different probabilities of obtaining recoveries of tagged fish in different areas of the Atlantic have not been estimated (International Commission for the Conservation of Atlantic Tunas 1993), so it is unclear how much weight should be given to tagging data.

The mitochondrial genome has many features that make it particularly suitable for genetic studies of intraspecific population structure, including its rapid rate of evolution, lack of recombination, and predominantly maternal inheritance (Avise et al. 1987; but see Gyllensten et al. 1991). Recently, the analysis of sequences obtained directly from mtDNA has become the tool of choice in studies attempting to resolve intraspecific phylogenies of haplotypes, and in determining molecular population structure within species (e.g., Quinn 1992; Wenink et al. 1993; Brown et al. 1993). In the swordfish, as in most vertebrate species (Brown et al. 1986; Wenink et al. 1994), a large portion of the variation in the mtDNA molecule is confined to two hypervariable segments of the noncoding control or D-loop region. This paper reports one of the first studies of intraspecific variation in the control region in fishes, and the first extensive study

Table 1. Collection details of the 50 swordfish assayed in this study.

Specimen[a]	Lat.	Long.	Region	Capture date	Sex[b]	Fork length[c] (cm)	Tissue/DNA isolation[d]	Type	Clade
18(PAC)	35°N	120°W	NE Pacific	30.09.90	F	na	G/CM	1	I
20(PAC)	35°N	120°W	NE Pacific	05.10.90	F	na	G/CM	2	I
6S(PAC)	35°N	120°W	NE Pacific	27.09.90	F	na	G/CM	3	I
11S(PAC)	35°N	120°W	NE Pacific	30.09.90	F	na	G/CM	3	I
13S(PAC)	35°N	120°W	NE Pacific	01.10.90	F	na	G/CM	3	I
5S(PAC)	30°N	115°W	NE Pacific	04.10.90	F	na	G/CM	4	I
8S(PAC)	35°N	120°W	NE Pacific	28.09.90	F	na	G/CM	5	I
16S(PAC)	30°N	115°W	NE Pacific	04.10.90	F	na	G/CM	31	I
80(MED)	35°N	00°E	Mediterranean	16.02.92	F	121	L/T	19	II
60(MED)	35°N	00°E	Mediterranean	16.02.92	F	191	L/T	20	II
48(MED)	35°N	00°E	Mediterranean	16.06.92	F	156	L/T	21	I
12(MED)	35°N	00°E	Mediterranean	8/1949	U	23	L/T	24	II
13(MED)	35°N	00°E	Mediterranean	4/1897	U	25	L/T	24	II
84(MED)	35°N	00°E	Mediterranean	16.06.92	F	165	L/T	26	I
91(MED)	35°N	00°E	Mediterranean	16.06.92	F	190	L/T	26	I
5(IBE)	35°N	10°W	NE Atlantic	27.11.92	F	189	L/T	7	I
4(IBE)	35°N	10°W	NE Atlantic	27.11.92	F	214	L/T	9	I
6(IBE)	35°N	10°W	NE Atlantic	28.11.92	F	190	L/T	12	I
5v(IBE)	35°N	10°W	NE Atlantic	27.11.92	F	99	L/T	12	I
26(MOR)	20°N	15°W	NE Atlantic	06.05.92	F	107	L/T	19	I
21(MOR)	20°N	15°W	NE Atlantic	06.05.92	F	185	L/T	21	I
8(MOR)	25°N	15°W	NE Atlantic	13.02.92	M	116	L/T	22	I
1(IBE)	40°N	10°W	NE Atlantic	13.02.92	M	158	L/T	23	I
7(MOR)	25°N	15°W	NE Atlantic	13.02.92	F	162	L/T	24	II
2(IBE)	40°N	10°W	NE Atlantic	13.02.92	F	155	L/T	25	II
3(IBE)	40°N	10°W	NE Atlantic	13.02.92	M	142	L/T	26	I
7(IBE)	35°N	10°W	NE Atlantic	27.11.91	F	227	L/T	30	I
1b(IBE)	35°N	10°W	NW Atlantic	27.11.91	F	225	L/T	31	I
11(CAN)	40°N	65°W	NW Atlantic	02.10.89	M	na	L/CM	9	I
15(CAN)	40°N	65°W	NW Atlantic	02.10.89	M	na	L/CM	10	I
19(CAN)	40°N	65°W	NW Atlantic	03.10.89	U	na	L/CM	11	II
21(CAN)	40°N	65°W	NW Atlantic	03.10.89	U	na	L/CM	12	I
22(CAN)	40°N	65°W	NW Atlantic	03.10.89	U	na	L/CM	12	I
1b(CAN)	40°N	65°W	NW Atlantic	07.09.90	M	109	H/EM	13	I
11b(CAN)	40°N	65°W	NW Atlantic	07.09.90	F	77	L/EM	14	I
14a(CAN)	40°N	65°W	NW Atlantic	08.09.90	U	na	H/EM	15	I
17(CAN)	40°N	65°W	NW Atlantic	08.09.90	M	89	BH/EM	16	I
25(CAN)	40°N	65°W	NW Atlantic	10.09.90	M	78	H/EM	17	I
26(CAN)	40°N	65°W	NW Atlantic	10.09.90	F	97	L/EM	18	I
1(CAN)	40°N	60°W	NW Atlantic	25.09.91	F	107	L/T	26	I
1(VIR)	40°N	70°W	NW Atlantic	26.05.65	U	32	F/T	27	I
2(AZO)	35°N	35°W	NW Atlantic	28.12.91	F	229	L/T	29	I
23(SAT)	35°N	20°W	S Atlantic	14.12.91	F	87	L/T	6	I
7(GUI)	00°S	15°W	S Atlantic	08.12.91	M	84	L/EM	8	I
21(SAT)	00°S	20°W	S Atlantic	14.12.91	F	185	L/T	32	I
1(GUI)	00°N	15°W	S Atlantic	07.12.91	M	178	L/EM	21	I
27(SAT)	00°S	20°W	S Atlantic	14.12.91	F	123	L/T	22	I
3(GUI)	00°N	15°W	S Atlantic	07.12.91	M	167	L/EM	33	I
28(BRA)	00°N	35°W	S Atlantic	29.12.91	M	152	L/T	27	I
29(BRA)	00°N	35°W	S Atlantic	29.12.91	F	191	L/T	28	I

[a](PAC), Pacific; (MED), Mediterranean; (IBE), Iberia; (MOR), Morroco; (CAN), Canada; (VIR), Virginia; (AZO), Azores; (SAT), South Atlantic; (GUI), Gulf of Guinea; (BRA), Brazil. Individuals from museum collections (AMNH 18960, USNM 48318, USNM 287998) are listed with the acronyms 12 (MED), 13 (MED) and 1 (VIR), respectively.

[b]F, female; M, male; U, unknown.

[c]na, not available.

[d]G, gonad; L, liver; H, heart; BH, brain heater; F, gill filament; CM, mtDNA obtained with cesium chloride gradients; T, total DNA; EM, enriched mitochondrial fraction.

in the swordfish. The main objective of this investigation is to determine whether swordfish in the Atlantic Ocean are structured into distinct phylogeographic populations, and secondarily to see if this structure corresponds to different stocks as delimited by the fishing industry.

Materials and methods

Populations sampled

A total of 50 swordfish were sampled mostly from three regions in the Atlantic Ocean (Fig. 1): the Northeast Atlantic (n = 18), the Northwest Atlantic (n = 13), and the South Atlantic (n = 8). The remainder of the samples came from the Pacific Ocean (n = 8) and from two localities in the Mediterranean Sea (n =7). Localities within the above regions were defined as a square, five degrees in longitude by five degrees in latitude, according to swordfish fisheries management conventions (International Commission for the Conservation of Atlantic Tunas 1993). Sampling details for each individual are given in Table 1.

DNA extraction

Fresh tissue from liver, heart, ovaries, or brain heater organ (Carey 1982) was obtained from 47 swordfish captured with longlines by commercial fishing vessels (Table 1). Unfortunately, this restricted opportunities to sample females from the few spawning grounds that are known presently. DNA was also extracted from three museum specimens preserved in formalin and alcohol in the collections of the American Museum of Natural History and the U.S. National Museum. The tissue used in these instances (e.g., 12/MED, Table 1) was gill filament to minimize damage to the specimens. For the majority of swordfish, total DNA was extracted using the protocol described in Sambrook et al. (1989); for some specimens mtDNA was extracted according to the protocols of Lansman et al. (1981) and Jones et al. (1989). Additional details are provided in Table 1.

Polymerase chain reaction amplification and sequencing

Five primers specifically designed for the control region of the swordfish were used to amplify the control region using polymerase chain reaction (PCR) (Saiki et al. 1985; 1988). Two primers were placed in the $tRNA^{pro}$ and $tRNA^{phe}$ genes flanking the control region: L15998-PRO (5′-TAC CCC AAA CTC CCA AAG CTA-3′) and H00585-PHE (5′-CAG TGT TAA GCT TTA ACT AAG CT-3′). L15998 was used with the internal primer H235 (5′-CGT GTG CAC TCT GAA ATG TCA-3′) to amplify an approximately 545 base pair (bp) segment, or with CSBDH (5′-TGA ATT AGG AAC CAG ATG CCA G-3′) to amplify roughly 380 bp of sequence. H00585-PHE was used in combination with primer L318 (5′-GTC ATT GAA GGT GAG GGA CA-3′) to amplify about 510 bp of sequence. PCR amplifications using this primer set were carried out in 25-μL reactions subjected to 35–38 cycles of 93°C for 1 min, 52°C for 1 min, 72° for 90 s, and a final extension at 72°C for 5 min. Product was run out on low melting point agarose, excised from the gel, and purified with the GeneClean Kit (Bio 101, La Jolla, Calif.). Sequencing was performed with the dideoxy chain termination method (Sanger et al. 1977) with Sequenase kits (USB). A detailed description of the sequencing conditions employed is given in Alvarado Bremer (1994). Using the fish-specific primer set, 630 bp of sequence of the control region were obtained for 40 swordfish. An additional 10 specimens were typed only for 280 bp of the L-domain because it contains 72% of all variable sites in the control region, and thus can be used to assign specimens to major mtDNA clades.

Phylogenetic analysis

Maximum parsimony analyses were conducted using the heuristic search algorithm of PAUP 3.1 (Swofford 1993). The swordfish was found to be very divergent from several istiophorids in their respective control region sequences (J.R. Alvarado Bremer, unpublished results). Thus, it was not appropriate to use an outgroup to root the trees (Avise et al. 1987). Instead, trees were rooted at the midpoint of the longest branch to facilitate the interpretation of the results, but all trees presented in this study should be considered unrooted. During the search, only minimal trees were kept, collapsing zero length branches. Starting trees were obtained by stepwise addition with swapping on minimal trees. Branch swapping was achieved using the tree bisection reconnection method, saving all minimal trees. To guard against suboptimal solutions arising from the input order of the haplotypes, we also conducted 10 searches using a random order of addition. Because a compression made the nucleotide at position 159 difficult to determine unambiguously, we deleted this position from the data set. Transversions and indels (insertions and deletions), which occur at about the same frequency, were weighted four times more than transitions to correct the substitutional bias favouring the latter (Alvarado Bremer 1994). Sequence analysis of the control region suggested that each of the three observed insertions or deletions involving tetramer motifs was produced independently by a single mutational event, and thus was weighted as such. Confidence limits to the branches were assigned by conducting 100 replicates of the bootstrap (Efron 1982).

A phylogeny of haplotypes was also constructed using the neighbour-joining method (Saitou and Nei 1987). Genetic distances among haplotypes were estimated using the maximum likelihood algorithm in PHYLIP (version 3.5; Felsenstein 1989). As in the maximum parsimony analysis, transversions and indels were given a weight four times larger than transitions, and the empirical frequency of residues was used.

Population structure

A hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to test several working hypotheses of stock population structure of the swordfish (International Commission for the Conservation of Atlantic Tunas 1993). Although the algorithm calls for a matrix of squared distances as the input matrix, a maximum likelihood estimate of genetic divergence was used instead because of the biased base composition in the sequences. This modification is not expected to alter the interpretation of the results (L. Excoffier, Department of Anthropology and Ecology, University of Geneva, 1227, Carougo, Switzerland, personal communication).

Table 2. Haplotypic diversity of the swordfish within the regions of capture.

Region	n	No. of haplotypes	Diversity (h)	Probability of identity
Northeast Pacific	8	6	0.91	0.22
Mediterranean	7	5	0.85	0.22
Northeast Atlantic	13	12	0.99	0.09
Northwest Atlantic	14	13	0.99	0.08
South Atlantic	8	8	1.00	0.12
Total Atlantic	35	27	0.98	0.05

Note: Diversity is given by $h = (1 - \Sigma x_i^2)n/(n - 1)$, where x_i is the frequency of a haplotype and n is the sample size (Nei and Tajima 1981). Probability of identity is given by the sum of squares of the frequencies (Vigilant et al. 1989).

In addition to calculating the amount of variance explained by any particular hierarchical arrangement, AMOVA performs several permutational procedures to test whether alternative combinations of populations or individuals are capable of explaining larger amounts of variation. Among-groups analysis tests whether the differences among regional groups are significant, assuming that the populations constituting the groups are real. Within-group analysis tests whether the differences among populations within a regional group are significant, assuming that regional groupings are real but that the populations within them are not. Among-populations analysis tests if the differences among haplotypes are significantly less within populations than among them. This test involves the random permutation of individuals among randomly chosen populations, holding sample sizes constant.

AMOVA incorporates distance between haplotypes in the calculation of haplotypic diversity at different hierarchical levels (among geographic regions, among populations within regions, and within populations). Haplotypic correlation measures are expressed as Φ statistics. Among regions, Φ_{CT} is defined as the correlation of random haplotypes within a group of populations, relative to that of random pairs of haplotypes drawn from the whole species. For the analysis among populations within regions, Φ_{SC} is the correlation of random haplotypes within populations, relative to that of random pairs of haplotypes from the region. Finally, for the within-population analysis, Φ_{ST} is the correlation of random haplotypes within populations, relative to that of random pairs of haplotypes drawn from the whole species (Excoffier et al. 1992). The Φ statistics thus quantify population structure at each level in a given hierarchy, analogous to the hierarchical F-statistics analysis of Cockerham (1969, 1973).

For the hierarchical analysis, swordfish were assigned to seven populations from five putatively independent stocks corresponding to the regions depicted in Fig. 1: (*i*) the Pacific Ocean, consisting of eight individuals collected in two neighbouring localities assigned to one population; (*ii*) the Northwest Atlantic, including two populations, one with five individuals sampled from Georges Banks, Nova Scotia, during the fall of 1989, and a second of seven individuals sampled in Georges Banks in the fall of 1990, one individual sampled west of 30°W near the Azores Islands, and another sampled off the coast of Virginia (see Table 1); (*iii*) the Northeast Atlantic, consisting of one population with 13 individuals captured off the coasts of the Iberian peninsula and Morocco; (*iv*) the South Atlantic, with eight individuals from three localities assigned to one population; and (*v*) the Mediterranean Sea, with seven individuals from two localities assigned to one population.

The extent of geographic heterogeneity in sample frequency distributions was also analyzed with a Monte Carlo simulation as described by Roff and Bentzen (1989), with the program MONTE in REAP (version 4.0) (McElroy et al. 1992). The level of heterogeneity in the original data matrix was assessed through chi-square analysis and compared with the value estimated from 10 000 randomizations of the original matrix. The probability associated with the estimated mean χ^2 value included values larger than or equal to the original chi-square. Comparisons of the distribution of types in the Pacific, Atlantic, and Mediterranean were conducted. The total Atlantic sample was divided into east and west Atlantic at 35°W, and this division was continued south of 5°N to increase sample sizes.

Results

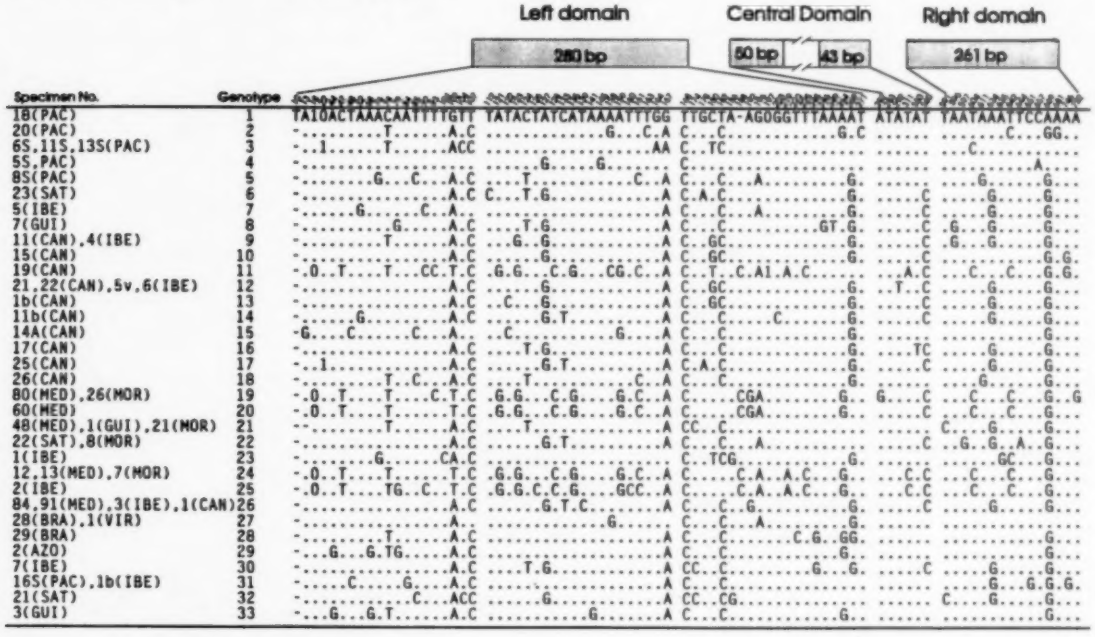
Variation in the control region

The control region of the swordfish was highly variable. Most of the variation was located in two hypervariable segments separated by a conserved central region (Fig. 2; see also Alvarado Bremer 1994). There were 81 variable sites in the control region of the 50 swordfish we sequenced. Sixty of these sites were contained in about 280 bp of sequence of the left domain, an additional 15 polymorphic sites were found in 262 bp of the right domain, and the remaining 6 sites were located in the 300-bp central domain (Fig. 2). Thirty-three mtDNA haplotypes were identified among 50 individuals. Very high values of haplotypic diversity (h) were estimated for all populations (Table 2). The probability that any two specimens drawn at random from the 35 Atlantic specimens differed in their control region sequences was 0.98. Lower values were obtained for the Mediterranean swordfish, but the diversity in this region was also high.

Phylogenetic analysis

Maximum parsimony analysis of the 33 mtDNA haplotypes generated two equally parsimonious trees 102 steps long. The most obvious feature of these trees is that two

Fig. 2. Variable sites in the control region segments of swordfish mtDNA. Numbers above sites refer to nucleotide position on the L-strand of the control region of the swordfish (see Alvarado Bremer 1994). Identity with the reference sequence is indicated by dots, and deletions of one residue with dashes. Three tetramer indels are indicated with a number 1 if residues are present, or with a 0 if they are absent. Nucleotide positions 18 and 22 correspond to the first residue of the repeat 5′-TACA-3′. Similarly, nucleotide 197 corresponds to the first nucleotide of indel 5′-CAAA-3′. Abbreviations of capture localities are as follows: AZO, Azores; BRA, Brazil; CAN, Canada; GUI; Gulf of Guinea; IBE, Iberia; MOR, Morocco; MED, Mediterranean; PAC, Pacific; SAT, South Atlantic; VIR, Virginia. Individuals listed in the same rows share the same sequences.



divergent clades were resolved, hereafter referred to as clades I and II. The input order of taxa did not affect the topology of the parsimony trees, as the same two minimal-length trees were always obtained. The trees differed mainly in some of the short basal branches of clade I. The information in the two trees was summarized in a strict consensus tree rooted at the midpoint (Fig. 3). The two major clades were supported by bootstrap values of 100%. Fourteen fixed differences distinguished haplotypes in these two clades (Fig. 2). These substitutions comprised one transversion, two indels, and 11 transitions, and occurred primarily in the left domain of the control region. One obvious shared feature of the haplotypes in clade I was the indel tetramer repeat 5′-TACA-3′ at nucleotide position 18. The mean divergence between haplotypes in the two clades was 3.8% (standard deviation (SD) 0.3); when corrected for the within-clade divergence of haplotypes this value (Nei 1987) is 2.7%.

Clade I grouped together 26 haplotypes found in 42 individuals collected in all regions sampled for this study. The mean divergence within clade I (excluding Pacific specimens) was 1.4% (SD 0.3). The branches at the base of clade I were extremely short, with most nodes supported by only one shared derived change, in marked contrast with the high number of uninformative substitutions (apomorphies) at the tips of the tree. An exception to this pattern was haplotype 3, which was supported by five derived changes. This haplotype was the most common haplotype in the Pacific sample, occurring in three individuals. Clade II was composed of five haplotypes (11, 24, 25, 19, and 20) that occurred in eight individuals (Fig. 3). The majority of these individuals came from the Mediterranean (n = 5) and the Northeast Atlantic (n = 2), but one individual possessing haplotype 11 came from Georges Banks. The mean divergence of haplotypes within clade II was 0.9% (SD 0.2).

The neighbour-joining tree calculated from maximum likelihood distances was very similar to the strict consensus tree from the parsimony analysis (Fig. 4). Differences between the parsimony and the neighbour-joining trees primarily involved groupings of haplotypes connected by very short basal branches of clade I, and also by minor rearrangements at the branch tips.

Analysis of population structure

The largest amount of variance (AMOVA) was accounted for with the Northeast Atlantic and South Atlantic populations in one regional group, the two Northwest Atlantic populations in another regional group, and the Mediterranean and Pacific regions as independent groups (Table 3). However, the amount of variance explained by the among-regions component was small (9.34%), suggesting that populations are not well structured. The amount of variation

Fig. 3. Strict consensus tree of the two most parsimonious trees, each 102 steps long. The tree summarizes the relatedness of 33 mitochondrial haplotypes of the swordfish. Two highly divergent clades of related haplotypes are identified with the Roman numerals I and II. Numbers below the nodes indicate the number of times a particular node is supported in 100-bootstrap replicates using the heuristic search option. Numbers at the tips of the branches correspond to the 33 haplotypes in Fig. 2, and the symbols refer to the geographical origin of haplotypes.

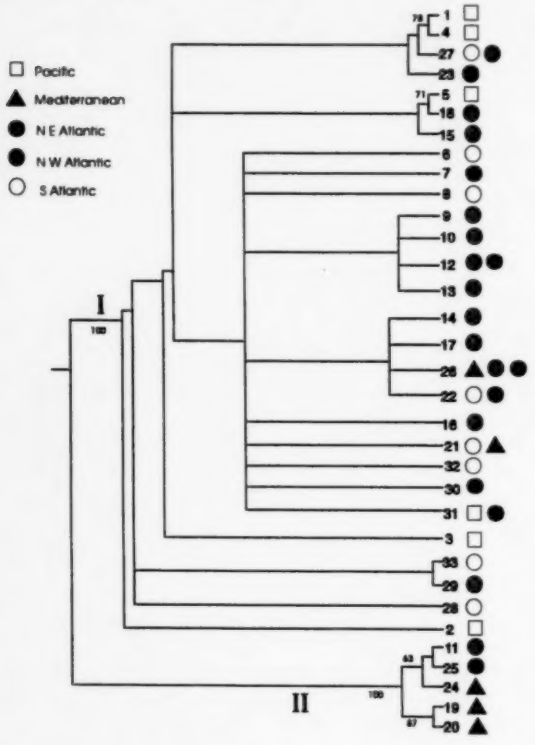


Fig. 4. Neighbour joining tree estimated from maximum likelihood values of divergence among 33 distinct mtDNA haplotypes. Symbols and numbers at the tip of the branches are the same as in Fig. 3. The tree was rerooted using clade II as the outgroup.

among populations within regional groupings was very small (2.04%) and not significant. Thus, the majority of the haplotypic diversity occurred within each population (88.62%). The probability associated with this statistic was highly significant, so it is very unlikely that the permutations of individuals across randomly selected populations, while holding sample size constant, would yield larger values of Φ_{ST} and σ_a^2.

The null distributions for Φ_{SC} and σ_b^2 suggest that it is possible to obtain larger values of variance if haplotypes are permuted among populations within the regional groupings. Eight alternative arrangements of regional groupings were attempted, since the null distributions of Φ_{CT} and σ_a^2 were markedly bimodal, indicating that the regional groupings were not optimal, and the permutation of populations among regional groupings could explain similar or larger amounts of variance. For instance, combining the Mediterranean with the Northeast Atlantic populations, and the South Atlantic with the two Northwest Atlantic populations, while keeping the Pacific population separate, gave a value of variance among populations slightly lower than the arrangement described above. This result is not surprising because the Northeast Atlantic population included three haplotypes corresponding to clade II, which typified the Mediterranean, whereas the South Atlantic and the Northwest Atlantic populations shared several closely related mtDNA haplotypes (Fig. 3). However, the largest amount of among-group variance was obtained by combining the 1989 Northwest Atlantic sample with the South Atlantic population, and the 1990 Northwest Atlantic sample with the Northeast Atlantic swordfish, while keeping the Pacific and the Mediterranean populations separate. This arrangement contrasts the Atlantic populations against the Pacific and the Mediterranean populations. The values of σ_a^2 increased to 15.58% ($P < 0.001$; 1000 permutations), but at the same time yielded negative values for σ_b^2 (−0.11%, $P = 0.767$; 1000 permutations). While this combination optimizes the amount of variation that distinguishes regional groupings, the negative values for the among-populations and within-group variance suggest that

Table 3. Hierarchical analysis of variance on the matrix of distances between swordfish haplotypes.

Variance component		Observed partition: Variance	% total	P^a	Φ statistics
Among regions	σ_a^2	0.0997×10^{-2}	9.34	0.0380	Φ_{CT}=0.093
Among populations and regions	σ_b^2	0.0217×10^{-2}	2.04	0.1828	Φ_{SC}=0.022
Within populations	σ_c^2	0.9455×10^{-2}	88.62	0.005	Φ_{ST}=0.144

[a]Probability of finding a more extreme variance component and Φ statistic than the observed values by chance alone.

Table 4. Probabilities associated with chi-square values for selected sample-pair comparisons of haplotype frequencies obtained using Monte Carlo simulations described by Roff and Bentzen (1989).

Sample	*n*	Mediterranean	Pacific	East Atlantic
Mediterranean	7	—		
Pacific	8	0.0495	—	
Total Atlantic	35	0.8841	0.0511	
East Atlantic	19	0.9254	—	—
West Atlantic	16	0.2223	—	0.5325

the individuals are more related between than within populations. This result underscores the large haplotypic diversity present in the Atlantic Ocean in the absence of phylogeographic population structure. One of the key findings of the AMOVA is that it identifies the Mediterranean population, with its large proportion of clade II individuals, as the most important in determining the amount of among-group variance that can be explained by any particular grouping of populations. Combining the Mediterranean with any region other than the Northeast Atlantic reduced the amount of among-group variation to less than 3%.

Table 4 presents results of Monte Carlo simulations (Roff and Bentzen 1989) used to analyze the extent of geographic heterogeneity in haplotype frequency distributions. The probability of obtaining mean chi-square values larger or equal to the observed values was significant for the interocean comparison between the Mediterranean and Pacific, and approached significance for the Atlantic and Pacific. However, the comparison of the total Atlantic sample with the Mediterranean was not significant. Similarly, haplotype frequency distributions were not significantly different between the east and the west Atlantic, or between these regions and the Mediterranean.

Discussion

Variation in control region relative to other mtDNA sequences

The swordfish is typical of other vertebrates with polymorphic mitochondrial control regions in that most variable sites are located in two hypervariable segments flanking the conserved central domain (Brown et al. 1986; Fajen and Breden 1992; Brown et al. 1993). Sixty of the 81 variable positions were located in a 280-bp segment in the left domain (Fig. 2). A survey of the mitochondrial ATPase 6 gene among 10 individuals detected four haplotypes and five (1.6%) variable sites in 300 nucleotides of sequence (Alvarado Bremer 1994). In contrast, analysis of 630 bp of the control region among the same 10 individuals identified nine distinct haplotypes defined by 47 variable positions. Thus, the control region is roughly five times more variable than the ATPase 6 gene.

The greater number of genetic markers located in the control region allows individuals to be assigned unequivocally to two divergent mtDNA clades, thus confirming this basic split suggested earlier by restriction analysis of the entire mitochondrial genome (Alvarado Bremer 1992, 1994) and by sequencing 300 bp of the ATPase 6 gene (Alvarado Bremer 1994). However, a survey of 612 nucleotides of the cytochrome *b* gene of six individuals from highly dispersed areas (Finnerty and Block 1992) revealed extremely low levels of variation (0.33%). This latter result is most likely a consequence of the small sample size assayed, which may have contained only individuals with clade I haplotypes, and the lower rates of substitution in this gene relative to the control region and the ATPase 6 gene.

Geographic structuring of populations

Swordfish with clade I haplotypes were found in all regions sampled (Table 1, Fig. 2). This clade includes some divergent haplotypes unique to the Pacific, as well as some closely related Pacific and Atlantic haplotypes (Fig. 3). In contrast, almost 60% of individuals with clade II haplotypes occurred in the Mediterranean Sea. Elsewhere these haplotypes were rare, and restricted to the North Atlantic. Only one of 15 (7%) individuals sampled in the Northwest Atlantic had haplotypes belonging to clade II. In the Northeast Atlantic, 2 of 14 (14%) individuals assayed had

clade II haplotypes. Migratory movements in and out of the Mediterranean were suggested recently by de la Serna et al. (1992), so that a higher frequency of swordfish with clade II haplotypes in the Atlantic region adjacent to the Mediterranean would be anticipated. Migration may also account for the appreciable number (40%) of individuals with clade I haplotypes in the Mediterranean sample. However, it is also possible that the Mediterranean breeding population consists of individuals belonging to both clades, implying their historical mixing.

Hierarchical analysis of molecular variance in control region sequences suggests that there are no appreciable differences separating the Pacific and Atlantic populations of the swordfish, but larger samples of Pacific individuals need to be analyzed to confirm this preliminary finding. Similarly, there is no significant differentiation among the stocks putatively associated with three of the Atlantic Ocean regions (Fig. 1). Most of the variance was contained within populations ($\sigma_c^2 > 88\%$), whereas about 9% was distributed among regions (Table 3). However, the analysis singled out the Mediterranean population in explaining most of the variance among regions, consistent with its high proportion of clade II haplotypes. When haplotypic composition was taken into account with the Roff–Bentzen test, only the Mediterranean sample was shown to be significantly different from the Pacific sample, although the difference between the Atlantic and Pacific samples approached significance.

The distinctiveness of the Mediterranean population of swordfish was also suggested by Magoulas et al. (1993) on the basis of a restriction fragment length polymorphism study of mtDNA haplotypes detected with two six-base cutter enzymes (*Eco*RV and *Pvu*II). They analyzed a Mediterranean sample of 223 individuals from Greece, Italy, and Spain, and Atlantic samples of 79 and 34 individuals from the Gulf of Guinea and an area west of Gibraltar, respectively. Four haplotypes were found in all areas, but with varying frequencies. For example, haplotype BB was present in only 7.6% of the Gulf of Guinea sample, while near Greece it reached a frequency of 40%. Conversely, haplotype AC was only present in 8.2% of the Greek sample, but occurred in 38% of the Gulf of Guinea individuals. Alvarado Bremer (1992, 1994) suggested previously that geographic structuring of mtDNA haplotypes in the North Atlantic population was the most likely explanation for the observed phylogenetic discontinuities revealed in the haplotype tree. In particular, specimen 19 (CAN) (Table 1 and Fig. 2) was markedly divergent from the other fish caught at Georges Banks. On the basis of the restriction fragment pattern for *Eco*RV and *Pvu*II, this clade II haplotype can now be assigned to the typically Mediterranean haplotype BB characterized by Magoulas et al. (1993).

In spite of the large amounts of variation that the control region harbours, the values of divergence that separate the Atlantic and the Pacific populations are relatively small. It is important to consider the time scale that gave rise to the current distribution of haplotypes, because the haplotypic composition of regional populations could be the product of current effects of gene flow, historical association of these two regions, or both. Although the North and South American landmasses may appear to effectively isolate the Pacific and the Atlantic populations (Nakamura 1985), current gene flow around the southern coast of Africa is possible. The presence of a seasonal fishery with high values of catch per unit of effort in the proximity of Cape Horn (Farber 1988) argues for significant interoceanic exchange. Because males and immature females are rare at such austral latitudes (Mejuto et al. 1992), gene flow must be primarily female mediated. Persistent gene flow over long periods may have been sufficient to nearly homogenize the haplotypic composition of the Atlantic and Pacific populations.

Evidence of historical gene flow between phylogeographically structured populations can be adduced from a gene tree by sister-group relationships of haplotypes from different regions (Slatkin and Maddison 1989). For example, sister-group relationships of haplotypes 5 and 18, haplotypes 1, 4, and 27, haplotypes 2, 28, 29, and 33, and haplotypes 11, 24, and 25 (Figs. 3 and 4) all suggest historical gene flow between the Pacific and Atlantic oceans. Very recent or current dispersal is indicated by the co-occurrence of haplotypes in the Pacific and Atlantic (haplotype 31), in the Atlantic and Mediterranean (haplotypes 21, 24, and 26), and in different regions of the Atlantic (haplotypes 12, 22, and 27).

Stock structure in the Atlantic Ocean

Several lines of evidence support the hypothesis that swordfish in the Atlantic Ocean comprise a single panmictic unit (Table 5). These include the homogeneous distribution of catch per unit effort for all age-classes, the widespread distribution of larvae, and the similar size distribution of fish captured throughout the Atlantic, in spite of historically different efforts for different areas. Additionally, levels of mtDNA haplotypic diversity for the total Atlantic Ocean are very high (0.98) and thus there is a low probability of identity of two randomly chosen haplotypes (Table 2).

While evidence in favour of the single-stock hypothesis for the Atlantic Ocean is substantial (Table 5), alternative explanations need to be explored, because in spite of the high mobility of swordfish (Taylor and Murphy 1992), no trans-Atlantic recaptures of tagged fish have ever been recorded. The absence of phylogeograhic association of mtDNA haplotypes within the Atlantic may be the result of seasonal sampling bias because a large proportion of individuals in this study came from feeding areas. For example, some migratory birds with high female natal philopatry are strongly structured geographically in their mtDNA haplotypes on the breeding grounds, but display no structuring on their winter feeding grounds because individuals from distinct lineages intermingle (Van Wagner and Baker 1990; Wenink et al. 1993). Dispersal power does not always predict how much gene flow actually occurs (Slatkin 1987; Wenink et al. 1993). Female swordfish may also be strongly philopatric to breeding areas, but may intermingle with other lineages on the feeding grounds throughout the Atlantic, or may disperse widely until they reach maturity. Phylogeographic structuring of breeding populations would only be revealed by analyzing early larval samples, or females with ripe ovaries. After hatching, passive dispersal of the larvae and early juveniles could be facilitated by strong oceanic currents (Tibbo and Lauzier 1969) and

Table 5. Hypotheses of stock structure of the Atlantic swordfish, with comments regarding support for various scenarios of population structure in the Atlantic and Mediterranean (adapted from International Commission for the Conservation of Atlantic Tunas 1993).

Stock structure	CPUE by age	Larval distribution	Catch distribution (JLL data)	Mark–recapture	Size distribution	Recruitment trends	This study
North Atlantic single stock	Yes	Yes	Yes	Yes	Yes	—	Yes[a]
Separate stocks							
NE vs. NW (boundary at 30°W)	No	No	No	[b]	No[c]	No[d]	No
Mediterranean vs. Atlantic	Yes	Yes	—		Yes	—	Yes[a]
Total Atlantic:							
N + S single stock	Yes	Yes	—	No[e]	—	—	Yes[f]

Note: CPUE, catch per unit of effort; JLL, Japanese longline data; NE, northeast; NW, northwest; N, north; S, south.

[a]There is large genetic similarity between Northeast and Northwest Atlantic. However, in the Northwest Atlantic there is a larger proportion of cluster II specimens, assigned in this study as of Mediterranean origin. Similarly, there appears to be movement of Atlantic forms into the Mediterranean.

[b]There have been no trans-Atlantic recoveries indicating E–W or W–E movement. However, movements have been demonstrated from central areas to the tropical west. Moreover, a common spawning stock in the tropical zone could be feeding NE–NW production units with slight communication between the two sides at the high latitudes. The differences in probability of obtaining recoveries in different east–west areas have not been carefully evaluated.

[c]If recruitment and exploitation are the same for NE and NW stocks, similar age composition would mask the existence of separate stocks. However, exploitation patterns appear to be somewhat different in the east and the west.

[d]In virtual population analysis, run separately for data collected in the east and in the west, recruitment trends were generally parallel for both stocks.

[e]No recovery from the North Atlantic has been reported in the south. Moreover, a common spawning population in the tropical area could be feeding the North and the South Atlantic. The different probabilities for obtaining recoveries in different areas of the Atlantic Ocean have not been taken into account.

[f]Representative samples for the South Atlantic come from the area just south of the arbitrary boundary at 5°N that delimits the North Atlantic and the South Atlantic, where most fishing by Spain is conducted. More southern areas need to be sampled.

their limited active dispersal capabilities (Alvarado Bremer 1994). Transoceanic movements are thus potentially possible and are intractable to tagging programs. Once the young swordfish become long-distance swimmers, subsequent dispersal can further homogenize the haplotypic composition of populations, making the Atlantic appear as a panmictic unit. This issue will not be resolved until haplotypic compositions of breeding populations are analyzed, and this is clearly a key requirement for informed management decisions in the future.

Antiquity of mtDNA lineages and phylogeography of the swordfish

The establishment of genetic discontinuities in several terrestrial vertebrates (Quinn 1992; Riddle et al. 1993; Wenink et al. 1993) as well as in freshwater fish (Bernatchez and Dodson 1991) has been attributed to geographic fragmentation produced by Pleistocene events. The influence of the glacial periods on the phylogeographic structuring of marine vertebrates is not well understood, but may have been important given that substantial changes in water temperatures and sea levels occurred during the Pleistocene. In the last half million years, there have been at least three long events that could have isolated the Mediterranean swordfish population. The most extensive glaciations caused the sea level to drop 115–120 m below the present level. During the Roman Regression, attributed to the Mindel Glaciation (400 000 years ago), the Strait of Gibraltar was dry, Sicily was joined by land to the Italian mainland, and the eastern part of the Mediterranean was connected with the western part by a narrow strait between Sicily and Africa (Nilsson 1982). Similar drops are suggested for the Riss Glaciation (approximately 130 000 – 170 000 years ago), followed by a transgression period that brought sea levels around 4–6 m above present sea level, ending about 80 000 years ago when a new eustatic period began. The most recent drop in sea level was 80–120 m, with the minimum level coinciding with the last glacial maximum about 22 000 years ago. The effective isolation of the Mediterranean population may have been possible even without the complete closure of the Straits of Gibraltar, owing to the swordfish's marked preference for water where depths are greater than 200 m.

Approximate dates of divergence of the two major clades of swordfish mtDNA haplotypes can in principle be estimated with a molecular clock, but the rate of molecular evolution of the mitochondrial genome in fishes is controversial. While earlier studies argued in favour of a retarded rate of substitution (Kocher et al. 1989; Thomas and Beckenbach 1989), recent studies suggest a similar rate to that for mammals (Brown et al. 1993; but see Martin et al. 1992; Martin and Palumbi 1993). A conservative estimate of 2% divergence per million years for the whole genome suggested for other vertebrates (Cann et al. 1987) can be used to calculate the approximate divergence time of clades I and II. Using the Kimura (1980) method to

correct for multiple substitutions at the same site, the average pairwise sequence divergence of the haplotypes in the two clades is 7.1% for the 280 bp of the hypervariable left domain. This compares with 1.1% divergence for the remainder of the mitochondrial molecule estimated with restriction fragment length polymorphisms. The hypervariable left domain in the control region therefore evolves at least 7.1/1.1 = 6.45 times as fast as the rest of the mitochondrial genome. Using this rate, at least 2 × 6.45 = 12.9 substitutional changes per million years would be expected for the left domain. Thus, divergence between clades is estimated to have taken place about 550 000 years ago, predating the Roman Regression by 150 000 years. Despite potentially large stochastic errors associated with these estimates, it seems reasonable to conclude that clade II haplotypes of the swordfish originated in the Mediterranean when it was effectively isolated by Pleistocene marine regressions.

Long-term effective size of the Atlantic population

Assuming that the Atlantic Ocean swordfish belong to one population, the long-term effective population size (N_e) of females can be estimated from the average sequence divergence in the hypervariable left domain of the control region of individuals belonging to clade I (excluding Pacific haplotypes). N_e can be estimated with the equation given by Wilson et al. (1985), as follows: $N_e = 10^6 t_x/sg$, where δ_x is the mean pairwise distance (corrected for multiple hits) among individuals, and $t_x = 0.5\delta_x$. Applying the estimated rate of evolution (s) for the left domain of 6.45%, an average generation time (g) of 6 yr (J. Mejuto, unpublished results), and an average sequence divergence of 3.5, N_e for the swordfish is about 45 000 females. Estimates of the current population size of mature females for the total Atlantic population are not available. For the North Atlantic, the earliest reliable estimates are those for 1978 (International Commission for the Conservation of Atlantic Tunas 1993), and the number of mature females can be estimated as roughly 80% of the total number of individuals classified as age 5 or older (277 000 × 0.8 = 221 600 mature females) in that year, a number that has subsequently been halved by overfishing. Because of similarites in catch per unit effort (an estimate of abundance), age-class composition, and total area, the size of the population in the South Atlantic can be estimated to be similar to that in the North Atlantic (J. Mejuto, unpublished results). Thus, up until very recently, the total number of mature females in the entire Atlantic Ocean must have been close to 500 000 individuals, which is more than 10 times the estimated historical female effective population size. This discrepancy probably arises from fluctuations in population size (especially population crashes), unequal sex ratios, age structure, and unequal family sizes. Nevertheless, the relatively high value of N_e suggests that the Atlantic swordfish population did not suffer any severe bottlenecks during the Pleistocene.

Patterns of population structure in cosmopolitan aquatic vertebrates

Specific characteristics of the biogeography, demography, and behaviour of species may be decisive in generating distinct phylogeographic patterns or categories (Avise et al. 1987). For instance, the Atlantic and Indo-Pacific populations of the green turtle are effectively isolated by landmasses because of the extreme oceanographic conditions around the southern tip of Africa. However, strong philopatric behaviour has produced a remarkable pattern of phylogeographic structuring within each ocean (Bowen et al. 1992). Conversely, interoceanic dispersal has been invoked to explain patterns of population genetic structure in some pelagic fishes. The current distribution of mtDNA haplotypes in the blue marlin is believed to be the result of secondary contact of lineages that initially diverged in allopatry in the Atlantic and Pacific, with unidirectional gene flow from the Indo-Pacific into the Atlantic around the southern tip of Africa (Finnerty and Block 1992). Similarly, one-way gene flow has been hypothesized to explain why mtDNA haplotypes belonging to one clade occur ubiquitously in Pacific and Atlantic populations of the sailfish (*Istiophorus platypterus*), whereas haplotypes of another clade are restricted to the Atlantic (Graves and McDowell 1992, 1995).

The patterns of differentiation described for the blue marlin and the sailfish contrast with the low levels of differentiation among the Pacific and Atlantic populations of the swordfish, but resemble the divergence of the Atlantic and the Mediterranean populations. Differences in physiology combined with historical oceanographic conditions may help explain the differences in geographic structuring of mtDNA haplotypes in swordfish relative to those in blue marlin and sailfish. Aside from phenotypic differences, the swordfish and these two istiophorid species differ in many physiological and behavioral aspects. The swordfish has a greater tolerance to cold water than any billfish species. Whereas the blue marlin prefers the warm mixed layer above the thermocline, rarely diving more than 200 m (Block et al. 1992*a*, 1992*b*), the swordfish makes daily feeding excursions to the surface at night and returns to near-freezing waters at depths around 600 m during the day. The low levels of genetic differentiation between Atlantic and Pacific populations of the swordfish suggest that this species was able to maintain gene flow at a time when the cold oceanographic conditions effectively isolated the Atlantic and Pacific populations of blue marlin and sailfish. All of these species are commonly typified as large, highly mobile pelagic fishes, and could be expected to display similarities in the structure of their mtDNA haplotypes. One caveat of this study is that superficial similarities in behaviour should not be used loosely to extrapolate to expected phylogeographic patterns of mtDNA from one species to another.

Acknowledgments

We thank O. Haddrath, D. Marshall, D. Stewart, M. Peck, V. Friesen, T. Birt, J.M. de la Serna, B. Garcia, M. Quintans, and K. Seymour for technical assistance, M. Vazquez Phillips and R. Motani for help with the illustrations, and Catherine Ayley for assistance with word processing and graphics. Tissue samples were obtained courtesy of J. Barlow and S. Kruse of the National Marine Fisheries Service, J. Porter and C. Dixon of the Department of Fisheries and Oceans (DFO) of Canada, and the Dixon family

of Woods Harbour, Nova Scotia. P.W. Wenink gave invaluble advice in primer design. C. McGowan and E.J. Crossman provided constructive criticisms that improved the manuscript. This study was supported in part by the National Research Council of Canada, grant A 9550, by a NSERC-DFO grant to C. McGowan, by IEO grant 04.03, and by a CONACYT-Mexico doctoral loan (49323) to J.R.A.B.

References

Alvarado Bremer, J.R. 1992. Stock differentiation of Atlantic swordfish using mitochondrial DNA analyses. International Commission for the Conservation of Atlantic Tuna, Madrid, Spain (ICCAT) Col. Vol. Sci. Pap. 31: 607–614.

Alvarado Bremer, J.R. 1994. Assessment of morphological and genetic variation of the swordfish (*Xiphias gladius* Linnaeus): evolutionary implications of allometric growth and of the patterns of nucleotide substitution in the mitochondrial genome. Ph.D. thesis, University of Toronto, Toronto, Ont.

Arocha, F., and D. Lee. 1993. Preliminary observations on sex ratio and maturity stages of the swordfish, *Xiphias gladius*, in the Northwest Atlantic. ICCAT Coll. Vol. Sci. Pap. 40: 425–432.

Avise, J.C., J. Arnold, R.M. Ball, E. Bermingham, T. Lamb, J.E. Neigel, C.A. Reeb, and N.C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18: 489–522.

Avise, J.C., R.T. Alisauskas, W.S. Nelson, and A.C. Davison. 1992. Matriarchal population genetic structure in an avian species with female natal philopatry. Evolution, 46: 1084–1096.

Ball, R.M.J., S. Freeman, F.C. James, E. Bermingham, and J.C. Avise. 1988. Phylogeographic population structure of the red-wing blackbird assessed by mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. 85: 1558–1562.

Bernatchez, L., and J.J. Dodson. 1991. Phylogeographic structure in mitochondrial DNA of the lake whitefish (*Coregonus clupeaformis*) and its relation to Pleistocene glaciations. Evolution, 45: 1016–1035.

Block, B.A., D. Booth, and F.G. Carey. 1992*a*. Direct measurement of swimming speeds and depth in blue marlin. J. Exp. Biol. 166: 267–284.

Block, B.A., D. Booth, and F.G. Carey. 1992*b*. Depth and temperature of the blue marlin, *Makaira nigricans*, observed by acoustic telemetry. Mar. Biol. (Berlin), 114: 175–183.

Bowen, B.W., A.B. Meylan, J.P. Ross, C.J. Limpus, G.H. Balazs, and J.C. Avise. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution, 46: 865–881.

Brown, G.G., G. Gadaleta, G. Pepe, C. Saccone, and E. Sbisa. 1986. Structural conservation and variation in d-loop-containing region of vertebrate mitochondrial DNA. J. Mol. Evol. 192: 503–511.

Brown, J.R., A.T. Beckenbach, and M.J. Smith. 1993. Intraspecific DNA sequence variation of the mitochondrial control region of white sturgeon (*Acipenser transmontanus*). Mol. Biol. Evol. 10: 326–341.

Burnett, C.D., J.S. Beckett, C.A. Dickson, P.C.F. Hurley, and T.D. Iles. 1987. A summary of releases and recaptures in the Canadian large pelagic fish tagging program 1961–1986. Can. Data Rep. Fish. Aquat. Sci. No. 673.

Cann, R.L., M. Stoneking, and A.C. Wilson. 1987. Mitochondrial DNA and human evolution. Nature (London), 325: 31–36.

Carey, F.G. 1982. A brain heater in the swordfish. Science (Washington, D.C.), 216: 327–1329.

Cavaliere, A. 1963. Studi sulla biologia e pesca di *Xiphias gladius* L. Nota II. Boll. Pesca Piscic. Idrobiol. 18: 143–170.

Cockerham, C.C. 1969. Variance of gene frequencies. Evolution, 23: 72–84.

Cockerham, C.C. 1973. Analysis of gene frequencies. Genetics, 74: 679–700.

Cooke, F., C.D. MacInnes, and J.P. Prevett. 1975. Gene flow between breeding populations of lesser snow geese. Auk, 92: 493–510.

de la Serna, J.M., E. Alot, and J. Mejuto. 1992. Analisis preliminar del sex-ratio por clase de talla del pez espada (*Xiphias gladius*) en el area Atlántica próxima al estrecho de Gibraltar. ICCAT Coll. Vol. Sci. Pap. 39: 514–521.

Efron, B. 1982. The jacknife, the bootstrap and other resampling plans. *In* Regional conference series in applied mathematics. Vol. 38. Society for Industrial and Applied Mathematics, Philadelphia, Pa.

Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131: 479–491.

Fajen, A., and F. Breden. 1992. Mitochondrial DNA sequence variation among natural populations of the trinidad guppy, *Poecilia reticulata*. Evolution, 46: 1457–1465.

Farber, M.I. 1988. An overview of available information pertinent to interpreting possible stock structure of swordfish in the Atlantic Ocean. ICCAT Col. Vol. Sci. Pap. 27I: 240–266.

Felsenstein, J. 1989. PHYLIP: Phylogeny inference package (version 3.2). Cladistics, 5: 164–166.

Finnerty, J.R., and B.A. Block. 1992. Direct sequencing of mitochondrial DNA detects highly divergent haplotypes in blue marlin (*Makaira nigricans*). Mol. Mar. Biol. Biotechnol. 1: 1–9.

Grall, C., D.P. de Sylva, E. Houde, and D. Houde. 1983. Distribution, relative abundance, and seasonality of swordfish larvae. Trans. Am. Fish. Soc. 112: 235–246.

Graves, J.E., and J.R. McDowell. 1992. Genetic differentiation of Atlantic and Pacific blue marlin and sailfish. *In* Proceedings of the 43rd Annual Tuna Conference, La Jolla, Calif., May 18–21. *Edited by* A.L.J. Coan and A.R. Jackson.

Graves, J.E., and J.R. McDowell. 1995. Inter-ocean genetic divergence of istiophorid billfishes. Mar. Biol. (Berlin), 122: 193–203.

Graves , J.E., S.D. Ferris, and A.E. Dizon. 1984. Close genetic similarity of Atlantic and Pacific skipjack tuna (*Katsuwanus pelamis*) demonstrated with restriction endonuclease analysis of mitochondrial DNA. Mar. Biol. (Berlin), 79: 315–319.

Gyllensten, U., D. Wharton, D. Josefsson, and A.C. Wilson. 1991. Paternal inheritance of mitochondrial DNA in mice. Nature (London), 352: 255–257.

International Commission for the Conservation of Atlantic Tunas. 1993. Report for biennial period, 1992–93. Part I (1992). International Commission for the Conservation of Atlantic Tunas, Madrid, Spain.

Jones, C.S., H. Tegelstrom, D.S. Latchman, and R.J. Berry. 1989. An improved rapid method for mitochondrial DNA isolation suitable for use in the study of closely related populations. Biochem. Genet. 26: 83–88.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. Mol. Evol. 16: 111–120.

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca, and A.C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. U.S.A. 86: 6196–6200.

Lansman, R.A., R.O. Shade, J.F. Shapira, and J.C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. J. Mol. Evol. 17: 214–226.

Magoulas, A., G. Kotoulas, J.M. de la Serna, G. de Metrio, N. Tsimenides, and E. Zouras. 1993. Genetic structure of swordfish (*Xiphias gladius*) populations of the Mediterranean and the eastern coast of the Atlantic: analysis of mitochondrial DNA markers. ICCAT Col. Vol. Sci. Pap. 40: 126–136.

Martin, A.P., and S.R. Palumbi. 1993. Body size, metabolic rate, generation time and the molecular clock. Proc. Natl. Acad. Sci. U.S.A. 90: 4087–4091.

Martin, A.P., G.J.P. Naylor, and S.R. Palumbi. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature (London), 357: 153–155.

Matsumoto, W.M., and T.K. Kazama. 1974. Occurrence of young billfishes in the central Pacific Ocean. *In* Proceedings of the International Billfish Symposium, Kailua-Kona, Hawaii, 9–12 Aug. 1972. *Edited by* R.S. Shomura and F.F. Williams. U.S. Department of Commerce, NOAA Tech. Rep. NMFS SSRF-675.

McElroy, D., P. Moran, E. Bermingham, and I. Kornfield. 1992. REAP: the restriction analysis package. J. Hered. 83: 157–158.

Mejuto, J., P. Sanchez, and J.M. de la Serna. 1992. Nominal catch per unit of effort by length groups and areas of the longline Spanish fleet targeting swordfish (*Xiphias gladius*) in the Atlantic, years 1988 to 1990 combined. ICCAT Col. Vol. Sci. Pap. 39: 615–625.

Nakamura, I. 1985. Billfishes of the world: an annotated and illustrated catalogue of marlins, sailfishes, spearfishes and swordfishes known to date. FAO Fish. Synop. 5: 65.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics, 97: 145–163.

Nilsson, T. 1982. The Pleistocene: geology and life in the quaternary ice age. D. Ridel Publishing Co., Dordrecht, Holland.

Quinn, T.W. 1992. The genetic legacy of Mother Goose: phylogeographic patterns of lesser snow goose *Chen caerulescens caerulescens* maternal lineages. Mol. Ecol. 1: 105–117.

Riddle, B.R., R.L. Honeycutt, and P.L. Lee. 1993. Mitochondrial DNA phylogeography in northern grasshopper mice (*Onychomys leucogaster*): the influence of Quaternary climatic oscillations on population dispersion and divergence. Mol. Ecol. 2: 183–193.

Roff, D.A., and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small sample sizes. Mol. Biol. Evol. 6: 539–545.

Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich, and N. Arnheim. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science (Washington, D.C.), 230: 1350–1354.

Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, and H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science (Washington, D.C.), 239: 487–491.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

Sambrook, J., E.J. Fritsch, and T. Maniatis. 1989. Molecular cloning. A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. 74: 5463–5467.

Slatkin, M. 1987. Gene flow and geographic structure of natural populations. Science (Washington, D.C.), 236: 787–792.

Slatkin, M., and W.P. Maddison. 1989. A cladistic measure of gene flow inferred from the phylogeny of alleles. Genetics, 123: 603–613.

Swofford, D.L. 1993. PAUP: phylogenetic analysis using parsimony, version 3.0r. Illinois Natural History Survey, Champaign, Ill.

Taylor, R.G., and M.D. Murphy. 1992. Reproductive biology of the swordfish *Xiphias gladius* in the straits of Florida and adjacent waters. Fish. Bull. U.S. 90: 809–816.

Thomas, W.K., and A.T. Beckenbach. 1989. Variation in salmonid mitochondrial DNA: evolutionary constraints and mechanisms of substitution. J. Mol. Evol. 29: 233–245.

Tibbo, S.N., and L.M. Lauzier. 1969. Larval swordfish (*Xiphias gladius*) from three localities in the western Atlantic. J. Fish. Res. Board Can. 26: 3248–3251.

Van Wagner, C., and A.J. Baker. 1990. Association between mitochondrial DNA and morphological evolution in Canada Geese. J. Mol. Evol. 31: 373–382.

Vigilant, L., R. Pennington, H. Harpending, T.D. Kocher, and A.C. Wilson. 1989. Mitochondrial DNA sequences in single hairs from a southern African population. Proc. Natl. Acad. Sci. U.S.A. 86: 9350–9354.

Wenink, P.W., A.J. Baker, and M.G.J. Tilanus. 1993. Hypervariable-control-region-sequences reveal global population structuring in a long-distance migrant shorebird, the Dunlin (*Calidris alpina*). Proc. Natl. Acad. Sci. U.S.A. 90: 94–98.

Wenink, P.W., A.J. Baker, and M.G.J. Tilanus. 1994. Mitochondrial control region sequences in two shorebird species, the turnstone and the dunlin, and their utility in population genetic studies. Mol. Biol. Evol. 2: 22–31.

Wilson, A.C., R.L. Cann, S.M. Carr, M. George, U.B. Gyllensten, K.M. Helm-Bychowski, R.G. Higuchi, S.R. Palumbi, E.M. Prager, R.D. Sage, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26: 375–400.

Groundwater and the selection of spawning sites by brook trout[1] (*Salvelinus fontinalis*)

R. Allen Curry and David L.G. Noakes

Abstract: Spawning areas selected by brook trout (*Salvelinus fontinalis*) displayed variable relationships to discharging groundwater across geologic regions. In Canadian Shield waters, spawning was associated with areas of distinct, discharging groundwater. The specific mechanism of selection could not be determined. Groundwater did not appear to influence the selection of individual redd sites within these discharge areas. Competition among females for the opportunity to spawn in a limited area defined by the discharging groundwater appeared to control the selection of redd sites. In southwestern Ontario streams, discharging groundwater was prominent throughout areas of spawning both at redds and at adjacent, nonspawning substrates (≤7 m). Consequently, relationships between groundwater and spawning site selection were ambiguous. On the unglaciated plateau of central Pennsylvania, no groundwater was observed in redds or nonspawning substrates in streams. Brook trout management programmes must consider these groundwater relationships and therefore the impact of land use on groundwater quality and quantity.

Résumé : On observe qu'il existe une variabilité au niveau du rapport entre les frayères utilisées par l'omble de fontaine, *Salvelinus fontinalis*, et l'émergence de l'eau souterraine d'une région géologique à une autre. Dans les eaux qui s'écoulent sur le Bouclier canadien, la fraie est associée à des secteurs d'émergence bien perceptible de l'eau souterraine. Nous n'avons pas pu déterminer le mécanisme précis de sélection des frayères. Il ne semble pas que l'eau souterraine influence le choix précis de nids de fraie dans ces zones d'émergence. La concurrence entre les femelles pour frayer sur une superficie limitée et définie par l'émergence de l'eau souterraine, semble contrôler le choix des nids. Dans les cours d'eau du sud-ouest de l'Ontario, il y a émergence d'eau souterraine partout dans les secteurs de fraie, tant là où sont les nids que dans les zones contigües (≤7 m). C'est pourquoi les rapports entre l'émergence de l'eau souterraine et le choix d'un lieu de ponte sont ambigus. Sur le plateau central de la Pennsylvanie, qui n'a pas été soumis à la glaciation, on n'a pas détecté l'émergence d'eau souterraine à l'endroit des nids de ponte ou sur les autres substrats non utilisés pour la fraie. Il importe de faire une place, dans les programmes d'aménagement de l'omble de fontaine, à ces rapports entre les lieux de fraie et l'émergence de l'eau souterraine; il faut donc penser aux répercussions de l'aménagement du territoire sur la qualité et la quantité d'eau souterraine.

[Traduit par la Rédaction]

Introduction

Early studies of brook trout (*Salvelinus fontinalis*) documented the presence of discharging groundwater in spawning habitats (Ricker 1932; White 1930). Continuing qualitative observations (Witzel and MacCrimmon 1983) and recent empirical studies (Fraser 1985; Gunn 1986) have suggested that successful, self-sustaining populations are dependent on the availability of spawning and incubation habitats in areas of groundwater discharge. However, a quantitative assessment of the relationship between spawning site selection and discharging groundwater has never been carried out.

We hypothesized that discharging groundwater is important for reproductive success by (*i*) providing cues that may direct trout to spawning areas, and (*ii*) controlling the selection of individual redd sites. We tested the predictions that the physical, thermal, and chemical properties of the interstitial waters of the redds would differ significantly from those of the surface water and other distant, nonselected, nearshore substrate waters (hypothesis 1), and from those of interstitial waters in the substrate immediately adjacent to, but not selected as, redds (hypothesis 2). The study had two components. The process study examined the spawning behaviour and groundwater

Received January 12, 1994. Accepted March 3, 1995.
J12234

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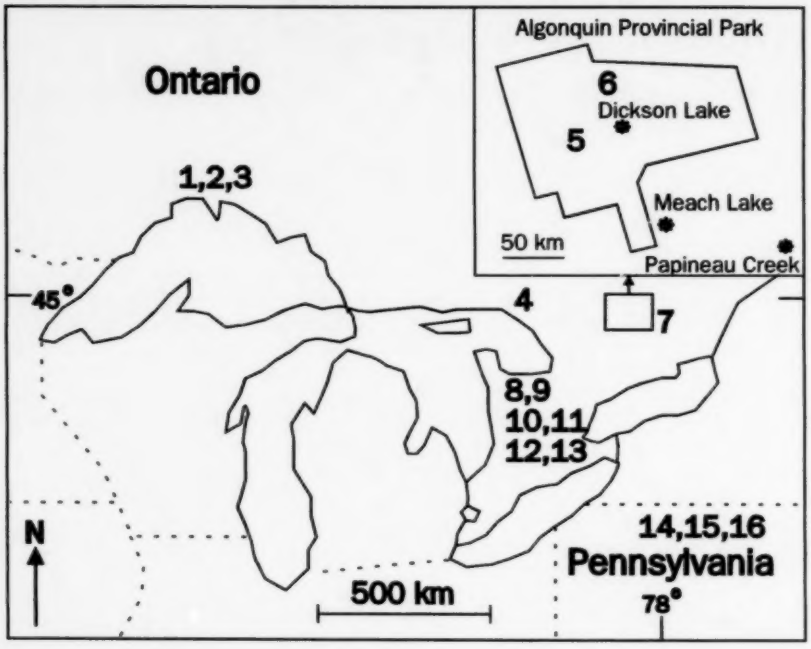
Fig. 1. Location of the three process study sites and 16 survey study sites.



discharge in detail at three sites in central Ontario. Because the occurrence and chemistry of groundwater varies across geologic regions (Freeze and Cherry 1979), we also conducted a survey study to examine spawning habitats from northern Ontario to central Pennsylvania to determine the applicability of the process study results across an extensive portion of the trout's native geographic range.

Methods

Study sites

Three process study sites were selected on the Canadian Shield (Fig. 1). Their waters were near neutral (pH 6–7) with specific conductance ranging from 40 to 60 $\mu S \cdot cm^{-1}$ (at 25°C). Dickson Lake (45°47′, 78°12′) had a surface area of 986 ha and mean depth of 8.5 m. Brook trout are native to Dickson Lake and no records of stocking exist. The spawning area was approximately 10 m^2, consisting of several sites 30–70 cm deep, 3–15 m offshore along 50 m of shoreline. Substrate in the spawning area consisted of variable combinations of sand, gravel, and cobbles. Adjacent areas were composed of silt and organic debris.

Meach Lake (45°18′, 78°20′; Fig. 1) consisted of a north and south basin (42 and 35 ha, respectively) joined by a 1–2 m wide channel. The maximum depths were 11 and 26 m in the north and south basins, respectively. Meach Lake was a natural brook trout lake, but stocking occurred annually from 1961 to 1986. The spawning area was located in the south basin where the lake narrowed between basins. The substrate in the narrows was dominated by silt and organic debris except at the spawning site where sand, gravel, cobbles, and several logs were present. The spawning site was 10 × 5 m, 30–70 cm deep, and 1–2 m offshore.

Spawning in Papineau Creek (45°10′, 77°48′; Fig. 1) occurred in a 50-m reach that varied from 5 to 10 m in width. It consisted of three sites that were 2 × 2 m, 10 × 3 m, and 4 × 2 m, 10–100 cm deep, and 1–5 m from the west bank. Substrate consisted of sand and gravel in variable proportions. No vegetation occurred in the spawning area. Brook trout are native to the stream, but stocking has occurred annually since 1974.

The distant, nonspawning areas were the closest nearshore areas accessible to the trout with depths, distances offshore, and substrate types (qualitatively determined) similar to those in the spawning areas. These nonspawning areas were approximately 100 m from the spawning areas at all process study locations.

The survey study examined seven spawning sites in lakes and streams on the Canadian Shield, six in streams on the glacial outwash plains of southwestern Ontario, and three in streams of the unglaciated plateau of central Pennsylvania (Fig. 1, Table 1).

Spawning observations

Spawning brook trout were observed at the process study sites in 1990 and 1991. Observations at each site began prior to spawning and continued until spawning was complete (Papineau Creek) or ice conditions prohibited access and observation in late November (Dickson and Meach lakes). The number of observations ranged from 8 to 14 per site annually.

Observations were made from shore at each site for periods ranging from 1 to 8 h. Redds were mapped and females and males recorded when present. Sexes were easily distinguished by behaviour and the prominent male

Table 1. Brook trout spawning sites examined across three geologic regions, Canadian Shield (CS), southwestern Ontario (SO), and Pennsylvania Plateau (PE) in the 1991 survey study.

Survey number	Region	Site	Date	Latitude	Longitude	Habitat type[a]	Redds sampled
1	CS	Jessie Lake (Nipigon River)	Oct. 28	49°18′	88°18′	L	2
2	CS	Lake Nipigon (South Bay)	Oct. 28	49°20′	88°18′	L	2
3	CS	Nipigon River (Parmachene)	Oct. 28	49°12′	88°18′	S	2
4	CS	Joe Lake	Nov. 1	46°30′	81°00′	L	2
5	CS	Redrock Lake	Nov. 6	45°30′	78°24′	L	2
6	CS	Charles Lake	Nov. 12	45°48′	78°24′	L	2
7	CS	Blairs Lake	Nov. 13	45°18′	77°36′	L	2
8	SO	Black Creek	Oct. 23	43°30′	80°06′	S	1
9	SO	Hanlon Creek	Oct. 23	43°36′	80°18′	S	2
10	SO	MacCrimmon Creek	Oct. 23	43°32′	80°18′	S	2
11	SO	Cranberry Creek	Nov. 25	42°48′	80°48′	S	2
12	SO	Kent Creek	Nov. 25	42°48′	80°18′	S	1
13	SO	Patterson Creek	Nov. 25	42°49′	80°18′	S	1
14	PE	Benner Run	Oct. 14	40°42′	78°48′	S	1
15	PE	Benner Run (Tramway)	Oct. 14	40°42′	78°50′	S	2
16	PE	Smays Run	Oct. 14	40°40′	78°50′	S	2

[a]S, stream; L, lake.

secondary sexual characteristics (Power 1980). Each redd was triangulated with two fixed markers on the shoreline.

The total length of each trout was estimated from objects of known length on the substrate. The estimate was validated by capturing and recording actual lengths of a portion of the observed trout. Temperatures were recorded in all redds with a YSI telethermometer (model 43TD) and remote thermistor (YSI model 406) inserted in a steel pipe (5 mm o.d., 10 cm length) connected to a 1.5-m wooden pole. Interstitial temperatures were recorded at a depth of 10 cm. The distant, nonspawning areas were sampled on November 11, November 5, and October 21 at Dickson Lake, Meach Lake, and Papineau Creek, respectively.

Superimposition of redds was determined by assuming redd diameters of 60 cm (Dickson and Meach lakes) and 40 cm (Papineau Creek) and observing overlapping circles within the spawning areas. The dimensions were derived from diameters observed at each site in 1990. Estimates of superimposition in the lakes were conservative because the last redds were not necessarily observed.

The survey study occurred in 1991. Spawning trout were observed for several hours and one or two redds being constructed were selected for analyses. Completed and unattended redds were only selected when they were clearly discernible.

Groundwater analysis

In the process study, five redds were selected when spawning was complete in Papineau Creek and near completion in Dickson and Meach lakes (the latest possible date with respect to access). Redds were selected to reflect (*i*) the range of female sizes, (*ii*) the timing of redd construction within the spawning period, and (*iii*) the spatial distribution of redds.

Mini-piezometers were installed in the centre of each redd (lakes) or the egg pocket (streams) following the methods of Lee and Cherry (1978). Piezometers were constructed of polyethylene tube (9.6 mm o.d., 6.4 mm i.d.). They were cut to desired lengths and the sampling end was slotted with seven semicircular notches (4 mm^2) over a 10 cm length located 5 cm from the piezometer end. The slotted section was wrapped twice with 1-mm nitex screen secured with duct tape. The end was sealed with a 3- to 5-cm silicone plug. Piezometers were bundled in pairs sampling substrate depths of 15–25 cm (depth of brook trout eggs, Snucins et al. 1992), and 35–45 cm (assumed depth where groundwater had not been altered by redd construction). Piezometers were allowed to discharge continuously for 24 h prior to sampling. Analyses in this study used the piezometers sampling 35–45 cm deep.

Similar pairs of piezometers were installed adjacent to, but outside each redd (equal to adjacent areas). In 1990, two pairs that were 50-cm onshore and separated by 50 cm were used. Interstitial waters were similar between redds and 50-cm onshore substrates, and so the distance to adjacent areas was extended to 100 cm in 1991. Three of five redds at each site had four piezometer pairs installed 100 cm adjacent to the redd centre; two pairs separated by 100 cm were located onshore and two pairs similarly separated were located offshore. Time limitations during sampling dictated a maximum number of piezometers per site; therefore, only one pair of piezometers was installed 100 cm directly offshore of the redd centre at the remaining two redds.

At the distant, nonspawning areas, three piezometers sampling 35–45 cm deep were installed and monitored in 1991. Sampling occurred at the time of redd sampling. Interstitial temperatures (10 cm depth) were recorded on November 11, November 5, and October 21 at Dickson Lake, Meach Lake, and Papineau Creek, respectively.

The hydraulic properties of the interstitial waters were determined using Darcy's formula for specific discharge (v, in $cm \cdot s^{-1}$):

Table 2. Percentage of superimposed redds on the basis of redd diameters of 60 cm in Dickson and Meach lakes and 40 cm in Papineau Creek.

		Number of superimposed redds						
	Year	0	1	2	3	4	5	6
Dickson	1990	61	25	8	2	2	2	0
	1991	54	30	10	6	0	0	0
Meach	1990	41	20	23	9	7	0	0
	1991	43	29	10	14	4	0	0
Papineau	1990	59	25	8	8	0	0	0
	1991	40	28	16	9	5	0	2

$$v = -K \times dh / dl$$

where K is the hydraulic conductivity ($cm \cdot s^{-1}$), dh is the hydraulic head differential (cm), and dl is the elevation head differential (cm). The dh was measured as the difference between observed pressure heads and surface water by using a metered rule or manometer (Winter et al. 1988). The dl was the depth of sampling within the substrate. The actual discharge from piezometers ($mL \cdot s^{-1}$) was used to calculate K following the procedures of Lee and Cherry (1978).

Dissolved oxygen (DO) concentrations were measured with a YSI DO meter (model 57). In 1990, the probe was inserted in a modified polyethylene bottle approximately 50 mm in diameter that was directly attached to each piezometer. In 1991, discharging water was collected in a 4-L, evacuated plastic bag attached to the piezometer. A second sample was slowly poured into a 500-mL graduated cylinder and the DO probe was inserted to the bottom of the cylinder and slowly agitated. Both methods and DO readings were calibrated with Winkler titrations. DO could not be measured when $v < 0.4 \times 10$ $cm \cdot s^{-1}$.

Specific conductance ($\mu S \cdot cm^{-1}$ at 25°C) or total ion concentration was measured in 1991 using a YSI SCT meter (model 33). A failure of the meter at the time of spawning resulted in a loss of data from Meach and Dickson lakes. Data were subsequently obtained on December 12 and February 5 at Meach and Dickson lakes, respectively. Ice had engulfed the nonspawning areas at Dickson Lake and no samples could be collected. Surface water temperatures and chemistry were sampled from the spawning area on every occasion of redd sampling.

In the survey study, a single piezometer was installed in the redd at depths greater than the female construction activity (10–25 cm depending on female size and stage of construction). A second piezometer was installed at a similar depth in the closest nonspawning area displaying a depth and surface substrate composition similar to that of the redd. The distance to nonspawning areas was 2–4, 1–7, and 0.5–4 m at sites on the Canadian Shield, in southwestern Ontario, and in Pennsylvania, respectively.

Data analysis

For the process study, differences among the surface water and interstitial waters of the redds and nonspawning areas were tested statistically with the nonparametric Kruskal–Wallis test ($\alpha = 0.01$; SAS Institute Inc. 1989; Sokal and Rohlf 1981). Statistical comparisons of redd and adjacent (process study) and redd and nonspawning area (survey study) interstitial waters were made using Wilcoxon paired-sample tests (SAS Institute Inc. 1989; Zar 1984). Groundwater was assumed to be moving in an offshore direction at the process study sites (Winter 1974); thus, when pairs of piezometers existed at a redd, they were combined into onshore and offshore pairs for analyses. To reduce the inflating error rate resulting from the multiple use of the redd data sets in these and subsequent analyses, significance levels were maintained at $\alpha = 0.01$. Similarly, statistical comparisons were minimized by using only specific discharge, DO, and specific conductance, and not comparing among sites or between years (process study). There were no statistical comparisons between nonspawning areas and surface waters, or among geographic regions in the survey study.

Although a sample size of five redds/site in the process study was small and could limit detection of significant differences when applying statistical models, this sample size represented 7–21% of the total redds in these populations, which is a biologically significant proportion of these populations (Curry 1993). Sample sizes were not increased, in an effort to minimize the impacts of our study on these populations.

Results

Process study

Spawning began first in Papineau Creek in early October and continued into late November at the lake sites. Overall, 24–67 redds, 18–44 females, and 69–170 males were observed at each site per year. Redds selected for study were constructed by females 25–45 cm in total length (TL) in the lakes and 14–31 cm TL in Papineau Creek. Among all sites 39–60% of redds were superimposed by one or more subsequent redds (Table 2).

The positive specific discharge and elevated specific conductance in interstitial waters of the redds indicated that groundwater was discharging through all redds at the three sites and in both years. Specific discharge was similar between years at each site (pooled in Fig. 2). No statistical differences were detected with distance from redds ($P > 0.01$), although specific discharge declined with distance on average. The distant (100 m), nonspawning area at Dickson Lake appeared similar to the spawning area, but two of its three measures were distinctly low ($<0.4 \times 10^{-3}$ $cm \cdot s^{-1}$).

Specific conductance was similar in redds and adjacent areas (1 m), and lower at distant (100 m), nonspawning areas and surface waters ($P > 0.01$; Fig. 2). Levels in redds and distant (100 m), nonspawning areas overlapped at Meach Lake.

Interstitial DO levels increased with distance from the redd and in surface waters at Dickson Lake and Papineau Creek ($P > 0.01$). At Meach Lake, DO levels were similar in redds, adjacent areas (1 m), and surface waters ($P > 0.01$). No levels could be measured at the distant (100 m), nonspawning areas because of low specific discharges.

Temperatures in all redds in both years ranged from 3.3 to 9.0°C (mean = 5.9°C, $n = 233$). The variation reflected

seasonal declines in surface water temperatures (mean = 5.3°C, range = 1.0–10.0°C, n = 284). Differences between redd and surface water temperatures ranged from −3.1 to 4.2°C (mean = 0.5°C, n = 239). No differences were detected between redds and adjacent substrates (1 m), or between redds and distant (100 m), nonspawning substrates ($P > 0.01$).

Survey study

Sampled redds were constructed by females ranging from 17 to 50 cm TL at the Canadian Shield sites, from 14 to 25 cm TL in southwestern Ontario, and from 12 to 15 cm TL in Pennsylvania.

At the Canadian Shield sites, the specific discharge of interstitial waters was not significantly different between redds and nonspawning areas ($P > 0.01$; Fig. 3). On average, it was greatest in the redds. Both interstitial DO and specific conductance levels in redds were similar to those in nonspawning areas and significantly less and greater than those in surface waters, respectively ($P < 0.01$). Temperatures were similar between redds and nonspawning areas ($P > 0.01$).

In the southwestern Ontario streams, differences in specific discharge were again not significant ($P > 0.01$; Fig. 3). Surface water downwelled into the streambed ($v < 0$) in one redd (site 8, Table 1). Differences in specific conductance, temperatures, and DO concentrations were not apparent between redds and nonspawning areas ($P > 0.01$) and redds and surface waters ($P > 0.01$). Substrate temperatures and DO levels were warmest and lowest of all regions.

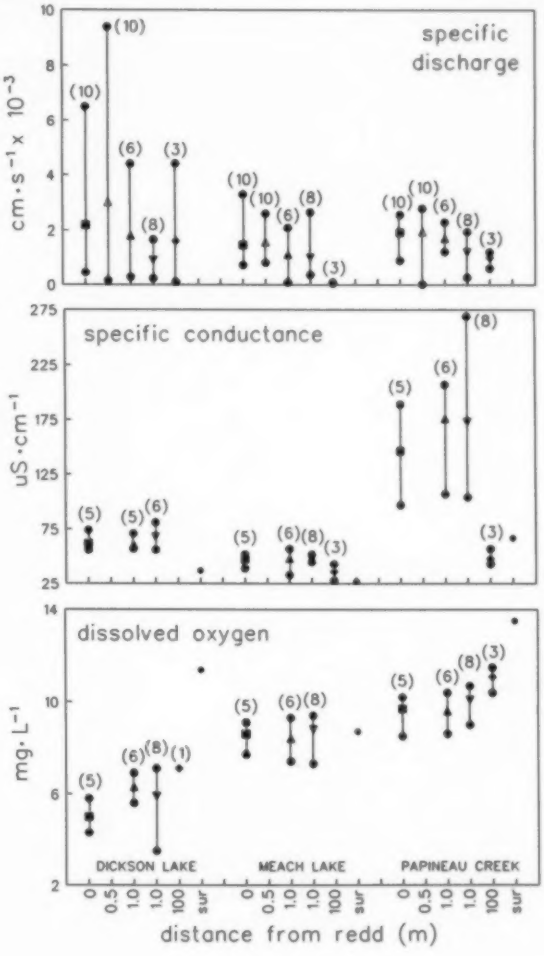
Specific discharge from redds and nonspawning areas was similar in streams on the Pennsylvania Plateau ($P > 0.01$; Fig. 3). One redd at site 15 (Table 1) exhibited downwelling surface waters. Specific conductance levels were also similar between areas ($P > 0.01$) and between interstitial redd and surface waters ($P > 0.01$). Temperatures were similar between areas, while DO concentrations appeared to be reduced in nonspawning areas.

Discussion

On the Canadian Shield, all spawning occurred in areas of distinct groundwater discharge in both lotic and lentic habitats. Individual redds and adjacent interstitial waters (≤1 m) where no spawning occurred generally displayed similar physical and chemical characteristics related to the discharging groundwater. Minor or no reductions in specific discharge were observed at nonspawning substrate 2–4 m from the redd centres (survey study). At 100 m from spawning areas, specific discharge of interstitial waters was statistically similar to that of redds, but reduced on average.

The consistent occurrence of discharging groundwater in spawning areas suggests that groundwater may have influenced the trout's homing to spawning areas. Upwelling groundwater can produce physical (flowing water), chemical, or temperature gradients in surface waters that could be used to search for spawning areas. Fraser (1982) introduced brook trout into a small lake on the Canadian Shield and later observed successful spawning over an area of upwelling groundwater despite the presence of an atypical spawning substrate (waterlogged sticks). Spawning brook

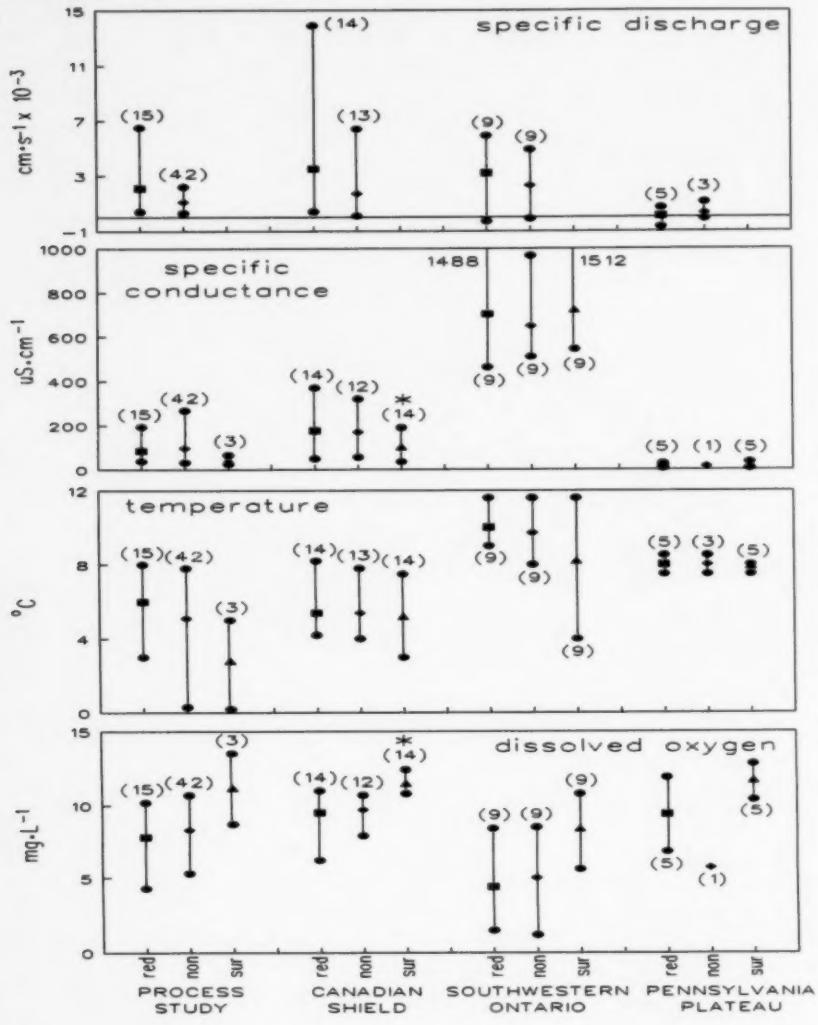
Fig. 2. Physical and chemical characteristics of interstitial waters in redds (0 m, ■), adjacent substrates (0.5 m, △; 1 m onshore, ▲; 1 m offshore, ▼), and distant, nonspawning substrates (100 m, ◆), and surface waters (sur, ○). Values are given as the mean and range. Sample sizes are given in parentheses. Samples were collected on the same day within sites; therefore, n = 1 for surface water samples. Specific discharges from redds in 1990 and 1991 were pooled. No significant differences from redds were detected.



trout are also known to be able to locate new areas of upwelling groundwater (Carline 1980; Webster 1962), suggesting that gradients created by discharging groundwater may be used for homing to spawning areas, at least during the early stages of invasions into new habitats.

Clearly the ability to detect gradients will be a function of their intensity as determined by the rate of groundwater discharge. However, the physical discharge of water alone does not appear to be a cue because specific discharge was similar between redds and distant, nonspawning areas at Dickson Lake and Papineau Creek. The use of temperature

Fig. 3. The physical and chemical characteristics of interstitial waters in redds (red, ■) and adjacent (1–7 m), nonspawning substrates (non, ◆), and surface waters (sur, ▲) in the survey study of 1991. Sample sizes are given in parentheses. *, significantly different from redds, $P < 0.01$.



gradients was also not suggested in this study. Chemical gradients may be important (Hara 1982), but apparently not oxygen, because no difference between redd and surface waters was observed at Meach Lake. Total ion concentrations were also similar between interstitial waters in the redds and distant, nonspawning area at Meach Lake, suggesting that ion concentration gradients alone are not involved in spawning site selection (Johnson and Webster 1977). Our study suggests that areas with both discharging and ion-rich water are used by brook trout for spawning in Canadian Shield waters.

The specific mechanisms of site selection remain to be determined. It is possible that the distinct ion concentrations observed in redds are unrelated to site selection. Trout may be using thermal plumes created by the groundwater and dispersed in streams by the flowing water (Lee and Hynes 1977) and in lakes by inlet streams or lake morphology, or chemical cues derived from conspecifics (Foster 1985; Keefe and Winn 1991) delivered to the surface water by the discharging groundwater. Alternatively, unknown cues unrelated to the groundwater may be involved (e.g., Smith 1985).

The absence of differences in interstitial water qualities between redds and adjacent nonredd substrates (≤1 m outside redd centres) suggests that trout do not use groundwater as a cue for selecting individual redd sites. Trout can detect discharge gradients (Webster and Eriksdottir 1976), but if minimum physical, thermal, and chemical thresholds are satisfied, then redd sites may be selected using visual or tactile

stimuli (e.g., substrate composition). Our observations of well-defined boundaries for spawning, substantial superimposition of redds (>38%), and the absence of relationships among female size, time of spawning, and redd habitat qualities (Curry et al. 1995) suggest that the selection of individual redd sites is regulated by competition for an opportunity to spawn in limited habitats in Canadian Shield waters.

In the southwestern Ontario streams, there were no physical and chemical differences among redds, nonspawning areas (1–7 m), and stream surface waters. These observations indicate the significant contribution of groundwater to the base flow in the vicinity of spawning areas. This suggests that groundwater may have drawn trout to generalized spawning areas, but it did not influence the selection of individual redd sites. Moreover, one traditional area of spawning where groundwater had previously discharged (Curry 1993) remained active despite a switch to downwelling stream water caused by a recently constructed beaver dam (White 1990).

Alternative stimuli such as depth, substrate, or cover may have been involved (Reiser and Wesche 1977; Witzel and MacCrimmon 1983), but differences in stream velocity (Smith 1973) between redds and nonspawning areas were not observed in the southwestern Ontario sites (Curry 1993). When discharging groundwater is prominent throughout the stream as in southwestern Ontario and as exists in streams in Michigan (Benson 1953) and Wisconsin (Carline 1980; Threinen and Poff 1963), warmer temperatures in redds than in nonredd substrates during the incubation period (Benson 1953) could impede ice formation in redds and therefore redd site selection. There is insufficient information in the present study to speculate on such relationships in these groundwater-dominated streams.

On the unglaciated plateau of central Pennsylvania, no groundwater was observed in redds or nonspawning areas. Discharge rates were also similar between these substrates. DO levels were greatest in the redds, which suggests its influence on spawning site selection in this region.

These observed differences in spawning site selection among the three regions can be explained by the geologic variability. The most common characteristics of spawning habitats in the present study, and for salmonids in general, are flowing, well-oxygenated waters in the interstices of redds. Such conditions were created on the Canadian Shield by the irregular rates of deposition of glacial outwash materials over exposed bedrock during the most recent glacier recessions. Pockets of variable compositions of glacio-fluvial materials and exposed bedrock were created across this region (Price 1973). Pockets composed of gravel–cobble substrate required for brook trout spawning and incubation (Snucins et al. 1992; Young et al. 1989) would also be linked to local groundwater regimes that produce discharging, ion-rich, oxygenated, and warmer groundwater in the nearshore zone (Freeze and Cherry 1979; Matthess 1982; Toth 1963). Pockets of distinctly discharging groundwater may have become a selection mechanism for successful reproduction as trout reinvaded Canadian Shield waters. The observed presence of ion-rich waters at spawning sites would also be similar to pre-Canadian Shield spawning and incubation habitats in the carbonate regions along the southwest to northeast routes of the receding glaciers (Gadd 1980; Terassmae 1986) and reinvading trout.

In southwestern Ontario, areas of groundwater discharge in streams are more abundant because of the dominance of glacio-fluvial deposits overlying a relatively uniform carbonate bedrock that dictates the geochemical similarity of surface and groundwaters. The dominance of discharging, local groundwater throughout these streams suggests that many suitable reproductive habitats would have existed for invading trout. Without distinct gradients related to discharging groundwater, selection of spawning sites would not necessarily become linked to the uniqueness of groundwater as was suggested in Canadian Shield waters.

The Pennsylvania Plateau was not glaciated during the most recent glacial epoch. This highland region has experienced an extended period of erosion and sediment removal that has reduced the depth of the unconsolidated overburden and limited the development of local groundwater systems. Brook trout that have been adapting to this environment would not have developed a dependence on discharging groundwater. The paucity of unconsolidated materials persists in the streams. The selection of spawning sites here is most likely controlled by the limited availability of suitable materials and surface water conditions for successful incubation (e.g., oxygen supply, stream velocity, and protection from freezing).

When groundwater was present, the observed relationships with brook trout suggest important management implications. In southwestern Ontario streams, a relationship between spawning site selection and groundwater may have been ambiguous, but the dominance of groundwater in these streams provides the necessary cold-water environments for juveniles and adults. Agricultural and urban development pressures are extreme in this region and much of the land has been cleared of its original forests. Trout habitats are restricted to headwater reaches of streams where forests and wetlands remain to produce groundwater-dominated habitats (Barton et al. 1985; Bowlby and Roff 1986; Witzel and MacCrimmon 1983). Protective measures for habitats need to acknowledge the significance of groundwater hydrology and development for trout in this region.

Similarly, in Canadian Shield waters, the link between groundwater and self-sustaining trout populations must be acknowledged. Spawning was observed to occur exclusively in areas of distinct groundwater discharge. Furthermore, there was substantial redd superimposition. Such results indicate that these populations are limited by the restricted availability of reproductive habitats as suggested by Benson (1953), Carline (1980), and Fraser (1985). A variety of resource development activities can alter groundwater hydrology and chemistry (e.g., forestry, Hartman and Scrivener 1990; agriculture, Barton et al. 1985; and water management, Curry et al. 1994). The impacts of such activities on groundwater regimes linked to brook trout reproductive habitats and the subsequent impacts on reproductive success remain unknown.

Acknowledgements

This project could not have been completed without the logistic and financial support of the Haliburton–Hastings and

Algonquin Fisheries Assessment Units, Ontario Ministry of Natural Resources (OMNR). Funding was also provided by a Department of Fisheries and Oceans science subvention grant, the Bancroft District (OMNR), a Sigma Xi research grant, and Natural Sciences and Engineering Research Council of Canada operating grant A6981 to D.L.G.N. Field assistance was provided by D. Haskins through the Environmental Youth Corp Programme, and C. Brady, R. Hawkins, F. Hicks, B. Irwin, and G. Morgan. R. Carline graciously facilitated sampling in Pennsylvania.

References

Barton, D.R., W.D. Taylor, and R.M. Biette. 1985. Dimensions of riparian buffer strips required to maintain trout habitat in Ontario streams. N. Am. J. Fish. Manage. 5: 364–378.

Benson, H.G. 1953. The importance of groundwater to trout populations in the Pigeon River, Michigan. J. North Am. Wildl. Conf. 18: 269–281.

Bowlby, J.N., and J.C. Roff. 1986. Trout biomass and habitat relationships in Ontario streams. Trans. Am. Fish. Soc. 115: 503–514.

Carline, R.F. 1980. Features of successful spawning site development for brook trout in Wisconsin ponds. Trans. Am. Fish. Soc. 109: 453–457.

Curry, R.A. 1993. Hydrogeology of brook charr (*Salvelinus fontinalis*) spawning and incubation habitats: linking aquatic and terrestrial ecosystems. Ph.D. thesis, University of Guelph, Guelph, Ont.

Curry, R.A., J. Gehrels, D.L.G. Noakes, and R. Swainson. 1994. Effects of streamflow regulation on groundwater discharge through brook trout, *Salvelinus fontinalis*, spawning and incubation habitats. Hydrobiologia, 277: 121–134.

Curry, R.A., D.L.G. Noakes, and G.E. Morgan. 1995. Groundwater and the incubation and emergence of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 52: 1741–1749.

Foster, N.R. 1985. Lake trout reproductive behaviour: influence of chemosensory cues from young-of-the-year by-products. Trans. Am. Fish. Soc. 114: 794–803.

Fraser, J.M. 1982. An atypical brook charr (*Salvelinus fontinalis*) spawning area. Environ. Biol. Fishes, 7: 385–388.

Fraser, J.M. 1985. Shoal spawning of brook trout, *Salvelinus fontinalis*, in a Precambrian Shield lake. Nat. Can. (Que.), 112: 163–174.

Freeze, R.A., and J.A. Cherry. 1979. Groundwater. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Gadd, N.R. 1980. Late-glacial regional iceflow patterns in eastern Ontario. Can. J. Earth Sci. 17: 1439–1453.

Gunn, J.M. 1986. Behaviour and ecology of salmonid fishes exposed to episodic pH depressions. Environ. Biol. Fishes, 17: 241–252.

Hara, T.J. (*Editor*). 1982. Chemoreception in fishes. Elsevier Scientific Publishing, Co., New York.

Hartman, G.F., and J.C. Scrivener. 1990. Impacts of forestry practices on a coastal stream ecosystem, Carnation Creek, British Columbia. Can. Bull. Fish. Aquat. Sci. No. 223.

Johnson, D.W., and D.A. Webster. 1977. Avoidance of low pH in selection of spawning sites by brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 34: 2215–2218.

Keefe, M., and H.E. Winn. 1991. Chemosensory attraction to home stream water and conspecifics by native brook trout, *Salvelinus fontinalis*, from two New England streams. Can. J. Fish. Aquat. Sci. 48: 938–944.

Lee, D.R., and J.A. Cherry. 1978. A field exercise on groundwater flow using seepage meters and mini-piezometers. J. Geol. Ed. 27: 6–10.

Lee, D.R., and H.B.N. Hynes. 1977. Identification of groundwater discharge zones in a reach of Hillman Creek in Ontario. Water Pollut. Res. Can. 13: 121–133.

Matthess, G. 1982. The properties of groundwater. J. Wiley and Sons, Inc., Toronto, Ont.

Power, G. 1980. The brook charr, *Salvelinus fontinalis*. *In* Charrs. Salmonid fishes of the genus *Salvelinus*. *Edited by* E.K. Balon. Dr. W. Junk Publishers, The Hague, Netherlands. pp. 141–203.

Price, R.J. 1973. Glacial and fluvioglacial landforms. Oliver and Boyd, Ltd., Edinburgh, U.K.

Reiser, D.W., and T.A. Wesche. 1977. Determination of physical and hydraulic preferences of brown and brook trout in the selection of spawning locations. *In* Water Research Series No. 64. Water Resources Research Institute, University of Wyoming, Laramie, Wyo.

Ricker, W.E. 1932. Studies of speckled trout (*Salvelinus fontinalis*) in Ontario. Ont. Fish. Res. Lab. Publ. 44: 69–110.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6. 4th ed. SAS Institute Inc., Cary, N.C.

Smith, A.K. 1973. Development and application of spawning velocity and depth criteria for Oregon salmonids. Trans. Am. Fish. Soc. 102: 312–316.

Smith, R.J.F. 1985. The control of fish migration. Springer-Verlag, New York.

Snucins, E.J., R.A. Curry, and J.M. Gunn. 1992. Brook trout (*Salvelinus fontinalis*) embryo habitat and timing of alevin emergence in a lake and stream. Can. J. Zool. 70: 423–427.

Sokal, R.R., and F.J. Rohlf. 1981. Biometry. 2nd ed. W.H. Freeman and Co., New York.

Terassmae, J. 1986. Some problems of late Wisconsin history and geochronology in southeastern Ontario. Can. J. Earth Sci. 17: 361–381.

Threinen, C.W., and R. Poff. 1963. The geography of Wisconsin trout streams. Wis. Acad. Sci. Arts Lett. 52: 57–75.

Toth, J. 1963. A theoretical analysis of groundwater flow in small drainage basins. J. Geophys. Res. 68: 4795–4812.

Webster, D.A. 1962. Artificial spawning facilities for brook trout, *Salvelinus fontinalis*. Trans. Am. Fish. Soc. 91: 168–174.

Webster, D.A., and G. Eriksdottir. 1976. Upwelling water as a factor influencing the choice of a spawning site by brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. 104: 416–421.

White, D.S. 1990. Biological relationships to convective flow patterns with stream beds. Hydrobiologia, 196: 149–158.

White, H.C. 1930. Some observations on the eastern brook trout (*Salvelinus fontinalis*) of Prince Edward Island. Trans. Am. Fish. Soc. 60: 101–108.

Winter, T.C. 1974. Numerical simulation analysis of the interaction of lakes and ground water. U.S. Geol. Surv. Prof. Pap. 1001.

Winter, T.C., J.W. Labaugh, and D.O. Rosenberry. 1988. The design and use of a hydraulic potentiomanometer for direct measurement of differences in hydraulic head between groundwater and surface water. Limnol. Oceanogr. 33: 1209–1214.

Witzel, L.D., and H.R. Maccrimmon. 1983. Embryo survival and emergence of brook charr, *Salvelinus fontinalis*, and brown trout, *Salmo trutta*, relative to redd gravel composition. Can. J. Zool. 61: 1783–1792.

Young, M.K., W.A. Hubert, and T.A. Wesche. 1989. Substrate alteration by spawning brook trout in a southeastern Wyoming stream. Trans. Am. Fish. Soc. 118: 379–385.

Zar, J.H. 1984. Biostatistical analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Groundwater and the incubation and emergence of brook trout (*Salvelinus fontinalis*)

R. Allen Curry, David L.G. Noakes, and George E. Morgan

Abstract: Groundwater was observed to discharge through brook trout (*Salvelinus fontinalis*) redds during the November to April incubation period in Canadian Shield waters. Groundwater was important for protecting redds from infiltrating surface water and ice. Physical and chemical characteristics of individual redd environments were similar during the incubation period. Substrate temperature and oxygen levels were reduced in nonredd substrates 100 cm from redds. The stability of discharge, temperature, dissolved oxygen, and specific conductance during incubation was similar between redd and nonredd substrates (≤100 cm away). The quality of redd habitats and embryo survival did not appear to be related to female competition for redd sites or time of spawning. There was evidence of density-dependent control of spawning success and the adaptive nature of alevin emergence behaviour in Canadian Shield waters.

Résumé : On a observé dans certains cours d'eau du Bouclier canadien un écoulement d'eaux souterraines dans des nids de fraie de l'omble de fontaine (*Salvelinus fontinalis*) lors de la période d'incubation, soit de novembre à avril. Cet écoulement d'eaux souterraines joue un rôle important pour protéger les nids contre les eaux de surface et la glace. Les caractéristiques physiques et chimiques des divers milieux de fraie étaient semblables durant la période d'incubation. La température et les niveaux d'oxygène étaient plus faibles dans les substrats situés à 100 cm des nids. La stabilité de l'écoulement, la température, l'oxygène dissous et la conductivité spécifique durant l'incubation étaient semblables pour les nids et pour les substrats avoisinants (à 100 cm maximum). On n'a pu observer de rapport entre la qualité des habitats de fraie et le taux de survie des embryons d'une part et la compétition des femelles pour les nids ou la période de la ponte d'autre part. Il semble exister un rapport entre la densité et le succès de la reproduction ainsi que l'adaptabilité du comportement de l'émergence des alevins dans les eaux du Bouclier canadien.

[Traduit par la Rédaction]

Introduction

Brook trout (*Salvelinus fontinalis*) select small, nearshore areas with discharging groundwater as spawning habitats in Canadian Shield waters (Curry and Noakes 1995). The groundwater regulates temperature, chemistry, and hydrology within the redd (Benson 1953; Fraser 1985; Gunn 1986). These physicochemical characteristics vary within a spawning area, but there are no absolute differences in groundwater characteristics between redd and adjacent (50–100 cm) nonredd substrates, and no relationship between groundwater qualities and competition for redd sites (Curry and Noakes 1995).

A stable incubation environment is critical for survival of embryos (Marten 1992; Peterson et al. 1977). Variations in the incubation environment may also affect alevin size and timing of emergence (Field-Dodgson 1988; Heming 1982), characteristics that influence individual fitness (Grant and Noakes 1987) and population dynamics (Elliott 1989). Groundwater was a dominant characteristic of spawning sites, and groundwater environments are typically stable during the several months of winter incubation (Freeze and Cherry 1979; Matthess 1982). This stability may be the characteristic important for successful reproduction.

In this study, we describe the physical and chemical characteristics of individual brook trout redds during the incubation and emergence periods. We predicted that if groundwater was important, then during these periods (*i*) the absolute qualities and stability of the physical and chemical environment would be greater in redds than in neighbouring, nonredd substrates, and (*ii*) quality and stability would be greatest in redds selected by the largest females (competitive advantage) and where embryo survival was maximized. In addition, time of spawning can influence reproductive

Received June 28, 1994. Accepted March 3, 1995.
J12456

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Table 1. Physical and chemical characteristics of redds, adjacent, nonredd substrates, and surface waters at three locations sampled in November, December, February, and April in 1990–1991 and 1991–1992.

Location	Specific discharge ($cm \cdot s^{-1} \times 10^{-3}$)			Temperature (°C)			Specific conductance ($\mu S \cdot cm^{-1}$)			Dissolved oxygen ($mg \cdot L^{-1}$)		
	Mean	Range	*n*	Mean	Range	*n*	Mean	Range	*n*	Mean	Range	*n*
Dickson Lake												
Redd	3.3	0.1–13.4	37	4.8	2.0–6.2	34	70	56–98	20	5.4	2.0–9.1	31
50 cm on	4.8	0.9–13.0	31	3.4	2.0–4.9	10	—	—	—	3.9	3.0–5.9	21
100 cm on	3.0	0.9–5.5	22	4.7	1.2–6.8	24	62	56–71	10	6.5	4.5–8.4	20
100 cm off	1.3	0.3–3.0	27	5.0	1.2–7.8	32	70	56–86	12	5.2	1.7–7.6	26
Surface water	na	na	na	1.8	−1.5–6.4	7	38	37–41	3	11.6	8.6–13.7	5
Meach Lake												
Redd	2.2	0.01–8.6	38	5.2	2.7–8.0	40	56	31–75	25	9.0	6.4–11.2	33
50 cm on	4.2	0.3–19.8	40	3.4	2.9–4.7	19	—	—	—	8.1	6.5–9.7	29
100 cm on	1.0	0.2–1.8	21	5.4	3.0–8.0	24	52	33–66	14	9.0	6.3–11.7	20
100 cm off	1.2	0.2–3.0	30	5.4	1.8–8.0	32	50	44–58	22	9.3	7.3–11.1	30
Surface water	na	na	na	2.3	0.0–7.9	6	35	27–43	5	9.5	8.0–11.7	5
Papineau Creek												
Redd	3.4	0.5–20.3	37	3.2	0.1–6.5	36	156	75–285	30	10.0	7.2–15.4	32
50 cm on	6.1	−7.4–45.2	36	0.9	0.4–1.7	10	—	—	—	8.7	6.8–12.1	26
100 cm on	2.8	0.6–9.7	22	3.2	0.0–6.0	24	162	61–226	23	10.0	8.3–12.8	23
100 cm off	4.2	0.1–51.9	32	2.3	−1.0–5.8	32	170	91–358	32	10.3	8.0–13.5	32
Surface water	na	na	na	1.2	−1.0–7.4	7	71	60–81	6	13.2	11.0–15.2	6

success (Hutchings 1991; Rombough 1985) and was examined in relation to habitat quality and stability. Groundwater may influence the chronology of emergence and size of alevins at emergence within populations (Curry et al. 1991; Snucins et al. 1992). We also observed emergence patterns and alevin sizes in individual redds to determine if groundwater was influencing these early life-history characteristics.

Methods

Brook trout spawning and incubation habitats were examined in the central Ontario, Canadian Shield waters of Dickson Lake (45°47′, 78°12′), Meach Lake (45°18′, 78°20′), and Papineau Creek (45°10′, 77°48′). The habitats were previously described by Curry and Noakes (1995). Details of the spawning ecology, including female characteristics and redd chronology, are given in Curry (1993).

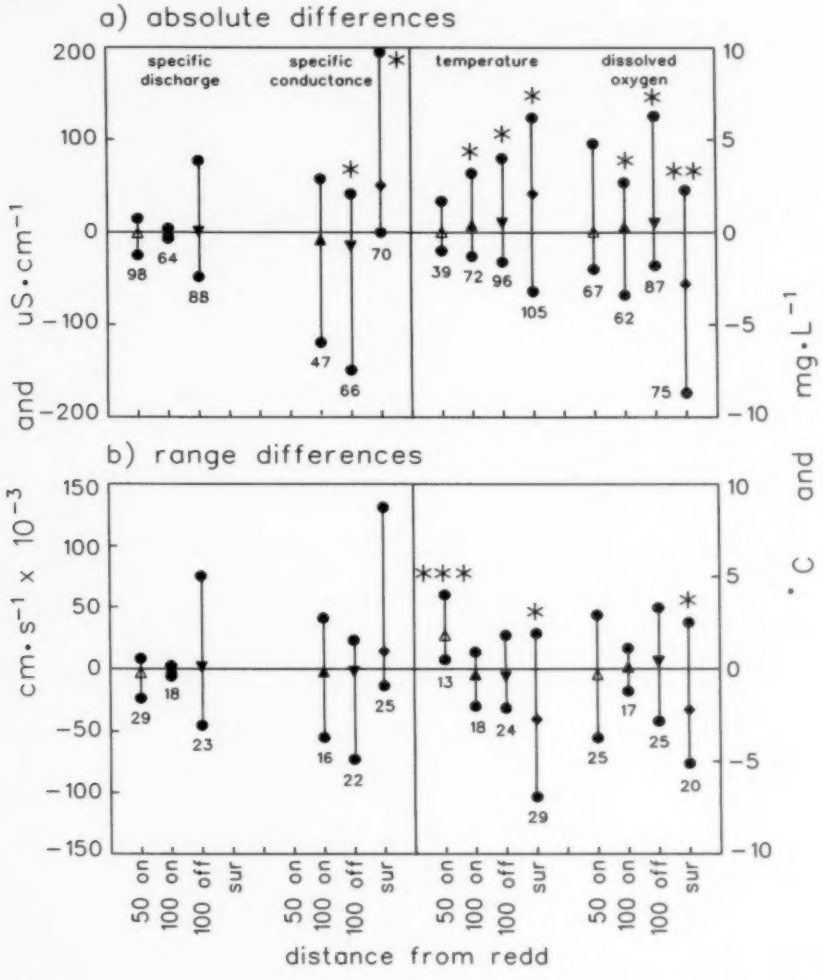
During the spawning seasons in 1990 and 1991, five redds at each location (7–21% of the total redds) were selected and installed with mini-piezometers (9.6 mm diameter) sampling at depths of 15–25 cm. Additional piezometers were installed in nonredd substrates adjacent to each redd. In 1990, two adjacent piezometers were installed 50 cm onshore of redds (100 cm separation); in 1991, two piezometers were installed 100 cm onshore and two were installed 100 cm offshore at three of five redds (100 cm separation of on- and off-shore pairs), and at the remaining two redds one piezometer was installed 100 cm offshore. The resulting number of adjacent substrate piezometers for each location and sampling period were as follows: 50 cm onshore = 10 (1990–1991); 100 cm onshore = 6 and 100 cm offshore = 8 (1991–1992). A maximum number of piezometers was dictated by winter sampling restrictions. Onshore and offshore sites were chosen to address the influence of the offshore flow of groundwater.

An additional three piezometers were installed at each location at distant (≈100 m), nonredd substrates displaying depths and substrate composition (qualitatively determined) similar to those of the spawning area. These piezometers sampled at depths of 35–45 cm to increase pressure heads and minimize surface ice damage.

Following the methods described in Curry and Noakes (1995), specific discharge ($cm \cdot s^{-1}$), dissolved oxygen concentration ($mg \cdot L^{-1}$), and specific conductance ($\mu S \cdot cm^{-1}$ at 25°C) were measured. Substrate temperature (°C) was measured at a 10 cm depth with a hand-held probe. Sampling occurred at the end of the spawning period (November), the embryo period (December), the free-embryo period (February), and the emergence period (April). All piezometers were sampled except when weather hampered the function of sampling equipment.

Statistical comparisons between redds and adjacent and surface water environments were made using paired *t* tests for absolute levels and ranges (maximum–minimum) used to define habitat stability ($\alpha = 0.01$; SAS Institute Inc. 1989; Sokal and Rohlf 1981). Differences were assumed to have normal distributions. Samples were pooled over years (redds) or sample sites (50 cm, 100 cm onshore, and 100 cm offshore) when relationships between redd and adjacent substrates were not suspected and verified with simple regression analyses. When relationships were apparent, statistical comparisons were made within locations (absolute levels of dissolved oxygen at 50 cm and specific discharge at 100 cm onshore; ranges in temperature at 50 cm). Statistical comparisons of redd and distant, nonredd environments

Fig. 1. Physical and chemical differences from redds at 50 cm onshore (△), 100 cm onshore (▲), 100 cm offshore (▼), and surface waters (◆) during the November to April incubation period at three locations in 1990–1991 and 1991–1992. Sample sizes are given. *, $P < 0.01$; **, $P < 0.01$ at Dickson Lake and Papineau Creek; ***, $P < 0.01$ at Meach Lake.



were not performed because of a paucity of data. Problems arising from an inflated error rate resulting from multiple use of redd data were assumed to be minimized by maintaining a low significance level.

Differences in the substrate environment among redds within locations were examined by ranking measured variables within periods and applying an analysis of variance (ANOVA) to the ranks ($\alpha = 0.01$). Ranking controlled the temporal variation among sampling periods. Variances (Cochrane's test, $\alpha = 0.01$) and residuals were examined. No transformations were required to meet these assumptions of ANOVA.

Evidence of female competition was sought by comparing the female total length ranked within the day of spawning with the absolute physicochemical levels ranked within each period and the ranked ranges over the incubation period. The influence of time of redd construction was similarly analyzed using time as the cumulative percentage of the total spawning period. Graphs were examined and relationships summarized with simple regression analyses (SAS Institute Inc. 1989). Ranking allowed pooling of samples among locations and between years.

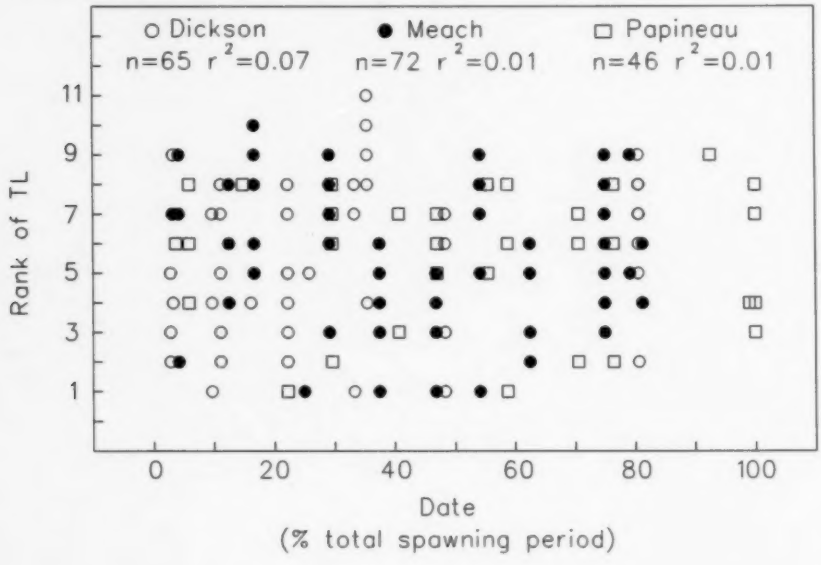
In Meach Lake in 1991–1992, emergence traps were installed over the redds with piezometers and one additional redd on March 15 ($n = 6$). The main section of the trap was a solid, dark plastic cylinder 40 cm in diameter with 8 cm sides. A mesh funnel (1 mm nitex) 20 cm long connected the trap to a removable 500-mL bottle with a one-way funnel and two mesh sides. Traps were examined every 2–5 days. The number of alevins that had emerged

Table 2. Correlations between female total length or time of redd construction (% total) and absolute levels and ranges in physicochemical characteristics in redds in November, December, February, and April, 1990–1991 and 1991–1992.

	Specific discharge				Temperature				Dissolved oxygen				Specific conductance			
	n	*P*	r^2	*m*	*n*	*P*	r^2	*m*	*n*	*P*	r^2	*m*	*n*	*P*	r^2	*m*
Absolute level																
Total length	112	0.54	<0.01	0.07	110	0.01	0.06	−0.30	96	0.22	0.02	0.15	75	<0.01	0.19	−0.54
Time (%)	112	0.10	0.03	0.01	110	0.52	<0.01	−0.003	96	0.73	<0.01	0.002	75	0.70	<0.01	−0.002
Range																
Total length	30	0.36	0.03	0.23	29	0.33	0.04	−0.24	28	0.32	0.04	−0.24	30	0.41	0.02	0.20
Time (%)	30	0.12	0.09	0.017	29	0.84	<0.01	0.002	28	0.08	0.11	−0.018	30	0.36	0.03	0.009

Note: Analyses used ranks within years and locations. *n*, sample size; *P*, probability; r^2, coefficient of determination; *m*, slope.

Fig. 2. Relationship between female total length (TL) ranked over the spawning period and time of spawning (%) at three locations in 1990 and 1991.



and their total lengths (nearest 1 mm), the hydraulic head in the redd (cm), the redd temperature (°C), the lake temperature (°C), and the depth (cm, fixed gauge) were recorded on each sampling date. The traps were removed on June 4 and the underlying substrate delimited by the trap was carefully excavated to a 25 cm depth and sieved (2 mm screen) to search for embryos and alevins. Only three alevins and no embryos were observed in one redd, which was excluded from all analyses.

Alevin size at emergence was compared among redds with an analysis of covariance (ANCOVA, $\alpha = 0.01$, Sokal and Rohlf 1981). Autocorrelation among dates of emergence in each redd was not apparent from the Durbin–Watson statistic and the first-order autocorrelation estimate (SAS Institute Inc. 1989). When differences were detected, a Tukey–Kramer test was applied ($\alpha = 0.01$, Day and Quinn 1989). Too few alevins were observed in redd 28 for it to be included in this analysis ($n = 8$).

Results

Specific discharge in the redds during the incubation period was always positive (upwelling), similar among locations (Table 1), and similar to that in adjacent, nonredd substrates (Fig. 1*a*). Its variability was the result of declines into midwinter and increases during the spring snowmelt period. One instance of downwelling surface water at the adjacent substrates was observed at Papineau Creek (Table 1). The greatest variability (Table 1) and range in variability within locations (Fig. 1*b*) occurred at 100 cm offshore, although differences from the redds were not statistically significant.

Specific conductance varied among locations with the greatest absolute levels and variability observed at Papineau Creek (Table 1). Levels increased at 100 cm offshore ($P < 0.01$) and were significantly less in surface waters than in redds ($P < 0.01$; Fig. 1*a*). Ranges in variability were similar between redds and adjacent substrates and surface waters (Fig. 1*b*).

Table 3. Physical and chemical characteristics of the redds and distant, nonredd substrates in December 1991.

		Specific discharge ($cm \cdot s^{-1} \times 10^{-3}$)		Temperature (°C)		Dissolved oxygen ($mg \cdot L^{-1}$)		Specific conductance ($\mu S \cdot cm^{-1}$)	
	n	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Meach Lake									
Redds	5	1.3±1.0	0.1–2.6	4.7±0.8	4.0–5.8	9.6±1.0	8.1–10.9	45±8	31–52
Nonredds	3	0.1±0.1	0.1–1.5	0.3±0.4	0.0–0.8	8.4±0.8	7.7–9.2	36±8	28–43
Surface water	1	—		0.2		11.7		27	
Papineau Lake									
Redds	5	0.9±0.5	0.5–1.7	2.4±1.2	1.0–4.0	12.6±1.6	11.1–15.4	129±42	76–164
Nonredds	3	0.9±0.6	0.2–1.4	−1.0±0.0	−1.0–1.0	13.4±0.6	12.8–13.8	43±5	38–46
Surface water	1	—		−1.0		15.9		76	

Temperature varied among locations and over the incubation periods (Table 1). It was reduced at 100 cm from that at the redd ($P < 0.01$) and in surface waters ($P < 0.01$; Fig. 1*a*). The range in variability was similar between substrates, except for a decrease at 50 cm onshore in Meach Lake ($P < 0.01$; Fig. 1*b*). The ranges were significantly greater in surface waters ($P < 0.01$; Fig. 1*b*).

Dissolved oxygen concentrations also varied among the locations (Table 1). At all locations except Meach Lake, dissolved oxygen levels decreased at 100 cm from the redd ($P < 0.01$) and were less than in surface waters ($P < 0.01$) (Fig. 1*a*). The range in variability was similar between redds and adjacent substrates and greater in surface waters ($P < 0.01$; Fig. 1*b*).

Statistically significant differences in substrate quality among redds within a location were not detected over both incubation periods (P values > 0.01). Female size was unrelated to the time of spawning (Fig. 2). Neither size nor time appeared to be related to absolute levels or ranges in the physicochemical characteristics of the incubation habitat (Table 2).

Difficulties with freezing temperatures and engulfing ice limited sampling of the distant, nonredd substrates to December in Meach Lake and Papineau Creek only. At Meach Lake, oxygen and conductivity levels indicated groundwater discharged from the nonredd site (Table 3). Its lower discharges were manifested in distinctly lower temperatures most similar to surface waters. At Papineau Creek, discharge was similar between redds and the distant, nonredd substrates, but lower conductances and temperatures similar to those in surface waters at the nonredd site indicated that the creek's discharging water originated from surface water entering the stream bed upstream (Table 3).

The total number of alevins captured varied from 8 to 180 per redd (Table 4). Few alevin mortalities were recorded (<5 per redd) and embryo mortalities ranged from 20 to 202 per redd. Egg to alevin survival estimates ranged from 6 to 68%. Egg to alevin survival did not appear to be associated with female size, time of spawning, or the absolute value and stability in the physical and chemical variables (Table 4). In redd 28, where survival was lowest (6%), temperature and specific discharge were also lowest.

The size of alevins at emergence varied within and among redds (Table 4). The smallest alevins were observed in the earliest spawned redd (14) and the largest alevins occurred in the latest (redd 46) (redd 14 = redd 23 < redd 40 = redd 46; $P < 0.01$). The patterns displayed no obvious relationships to female size or the physicochemical characteristics of the incubation environment. Alevin size within a redd decreased with date of emergence (slopes = −0.01 to −0.13).

The period of alevin emergence began at the end of March and continued until the end of May, ranging from 31 to 62 days/redd (Fig. 3). Individual females spawned for <5 days within a season. Mean daily emergence did not appear to be associated with changes in lake levels or temperatures. Peak emergence coincided with an increase in groundwater discharge at the end of April in redds 14 and 23. No peak was observed in redd 40. In redd 46, an early peak (maximum 30 alevins/day), a period of zero emergence, and a later period of 1 alevin/day were observed. The majority of alevins emerged prior to the maximum increase in groundwater discharge. On March 29, 30 alevins did emerge coincident with a sharp decline in groundwater flow. These patterns of emergence from each redd appeared to be unrelated to the time of spawning, female size, or the physicochemical nature of the incubation habitat (Fig. 3, Table 4).

Discussion

We observed that upwelling groundwater present in brook trout redds at the time of spawning (Curry and Noakes 1995) persisted throughout incubation. This continuously flowing groundwater provided thermal and oxygen regimes that exceeded critical minima for successful incubation (Garside 1966; Hokanson et al. 1973). Both temperature and dissolved oxygen levels were reduced in substrates 100 cm from the redd. These characteristics were not apparent at the time of spawning (Curry and Noakes 1995) and suggest the longer term significance of the redd construction process. The groundwater flow would also be important for removing metabolites from the incubation environment (Hayes et al. 1951; Silver et al. 1963) and protecting redds

Table 4. Date of spawning, female total length, alevin emergence characteristics, and the physical and chemical characteristics during the incubation period (November–April, $n = 4$) in five brook trout redds in Meach Lake, 1991–1992.

	Redd									
	14		23		28		40		46	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Spawning date	Nov. 8		Nov. 14		Nov. 14		Nov. 19		Nov. 19	
Female total length (cm)	39		25		25		39		31	
Dead embryos	160		202		127		64		20	
Dead alevins	2		4		0		0		4	
Emerged alevins	180		122		8		38		51	
Egg–alevin survival (%)	53		37		6		37		68	
Alevin total length (mm)	21±2		22±2		22±2		24±2		24±1	
	$n = 114$		$n = 122$		$n = 8$		$n = 38$		$n = 51$	
50% emergence (days)	185		172		182		149		131	
Duration of emergence (days)	62		47		31		41		59	
Specific discharge ($cm \cdot s^{-1} \times 10^{-3}$)	1.8±0.4	1.3–2.2	2.2±0.3	2.1–2.6	0.8±0.5	0.3–1.4	—		1.8±0.9	1.2–3.0
Temperature (°C)	6.0±0.2	5.8–6.2	5.8±0.9	5.0–7.0	5.1±0.8	4.0–5.5	5.2±0.5[a]	4.8–5.5	5.4±0.7	4.6–6.0
Dissolved oxygen ($mg \cdot L^{-1}$)	8.7±0.9	7.7–9.8	10.1±1.0	9.0–11.0	9.8±0.5	9.4–10.6	—		9.8±0.6	9.4–10.7
Specific conductance ($\mu S \cdot cm^{-1}$)	56±5	51–59	43±10	31–51	56±3	52–58	—		46±6	40–51

[a] $n = 2$ (Nov. 19 and April 8).

from the freezing or infiltrating cold surface waters that were observed in the distant, nonredd substrate environments without upwelling groundwater.

Groundwater appears to protect incubation habitats from freezing and may have been important for recent brook trout evolution at these locations. In cold climate regions, surface ice can extend into the substrate (Franzin and Harbicht 1985; McCart and Bain 1974; Needham and Jones 1959). Groundwater remains warmer than surface waters during winter (Matthess 1982) and restricted ice development at discharge points does provide habitats for free-swimming brook trout (Cunjak and Power 1986). Brook trout reinvading northeastern North America after the last glacial epoch may have been restricted to water bodies with discharging groundwater that could be used for spawning, incubation, and free-swimming young and adults.

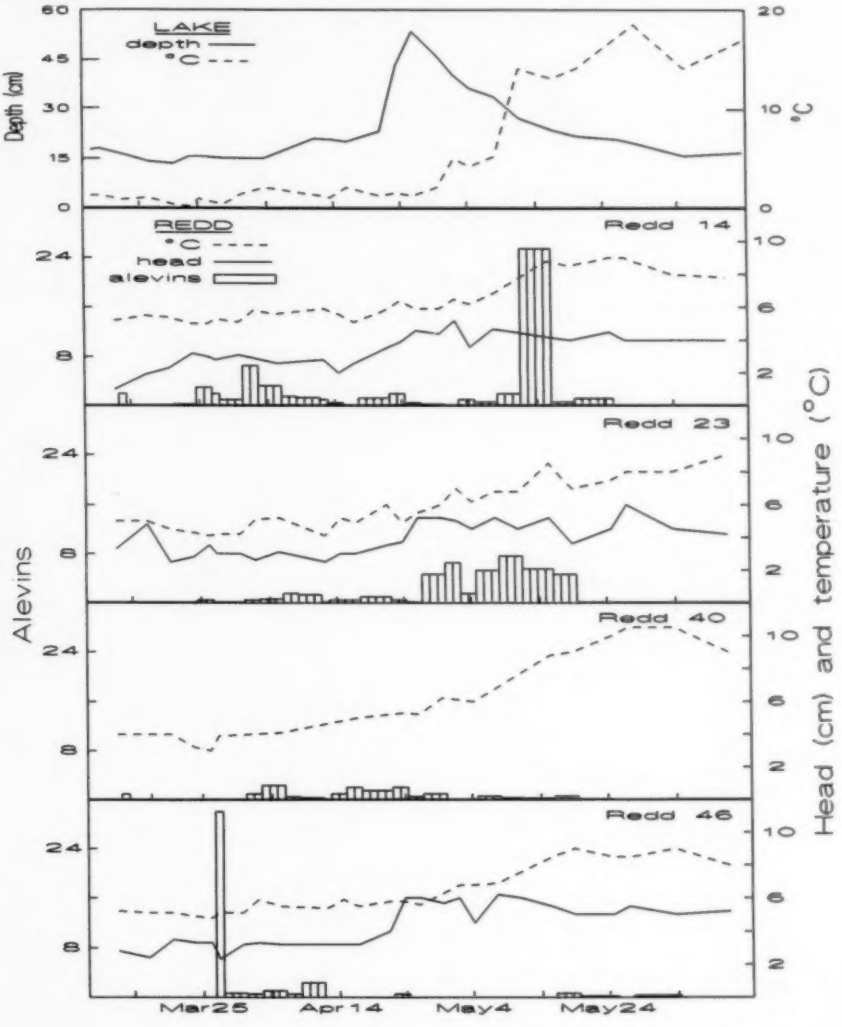
Within the relatively small areas of upwelling groundwater, it is not clear if groundwater cues are used by females when selecting redd sites (Curry and Noakes 1995). Physical and chemical differences among redds exist at the time of spawning and during incubation, but the differences selected by spawning females did not persist through the incubation period and appeared to be unrelated to competition among females for redd sites. Similarly, there was no indication that redd environments were more stable than adjacent, nonredd substrates. Such lines of evidence combined with the observed aggressive interactions among females during spawning (Curry 1993) and the relatively small size of spawning and incubation habitats indicate that spawning success is density dependent in these populations, i.e., mature females compete for an opportunity to spawn in limited habitats (Carline 1980; Fraser 1985).

The observed survival rate from the time of egg deposition until the free-embryo stage of development agrees with Fiss and Carline's (1993) median survival rate to the free-embryo stage of 16–86 % in individual, natural redds. Our lowest survival rate was observed in the redd with the least groundwater discharge (and substrate permeability, Curry 1993), which suggests a positive influence of groundwater on survival when temperature and oxygen are not limiting (Hausle and Coble 1976; Witzel and MacCrimmon 1983; Sowden and Power 1985). Testing of predictions from this hypothesis will require more observations from individual brook trout redds.

The emergence of salmonid alevins to a free-swimming form is a critical life-history transition period defined by a change in feeding from endogenous to exogenous resources, and a dramatic change in habitats. Consequently, the timing of emergence from the gravel has very important implications for survival of individuals and populations (Bams 1969; Brannon 1987; Elliott 1989; LeCren 1962; Noakes 1989). It may be more critical than egg–alevin survival for determining overall reproductive success.

The cues used by brook trout alevins to initiate emergence are not fully understood. Physiological development brings alevins to a stage where emergence can occur, but it is not the stimulus for the behaviour (Heming 1982). Emergence of brook trout alevins has been associated with the spring increase in groundwater discharge (Curry et al. 1991; Snucins et al. 1992). It was unclear if a similar

Fig. 3. Lake temperatures and levels and mean number of alevins emerging per day, hydraulic head, and substrate temperature in four brook trout redds in Meach Lake during the spring of 1992.



relationship existed in Meach Lake where variable patterns of emergence were observed in relation to groundwater discharge. A further complicating factor is the possibility of free-embryo or alevin movements within the redd (Dill and Northcote 1970; Nunan and Noakes 1985), which suggests that they can select alternative habitats to those originally chosen by spawning females. The potential impacts of free-embryo and alevin social behaviours on the timing of emergence are also unknown.

The observed protracted emergence period appears to capitalize on a diversity of survival tactics for alevins and young-of-the-year. Early emergence potentially provides a growth advantage as suggested by the wide range in size of young-of-the-year (Curry et al. 1993; Grant 1990). This could translate to greater first-winter survival of larger individuals (Gardiner and Geddes 1980; Hunt 1969). However, delaying emergence results in a smaller body size, and early emergence prior to the peak spring flood in lotic habitats may result in mortalities or displacement from the population (Curry et al. 1991). Food resources are linked to seasonal conditions with peak production most likely coinciding with the loss of ice cover and increasing spring temperatures (Wetzel 1983). This may be the best time for emergence. We found no evidence that such environmental cues, e.g., groundwater discharge or lake levels, were linked to emergence, although the cues may exist within the microhabitat of individual alevins in the substrate. The available evidence suggests that the extended emergence

period within redds enhances the individual fitness of females by ensuring survival of some proportion of their alevins despite annual variability in environmental conditions and therefore the best time for emergence. This tactic also ensures a variability of genotypic characteristics within and among year-classes, which would strengthen population and overall evolutionary fitness.

Such hypotheses do not consider the importance of other life-history tactics for reproductive success. The size characteristics of emerging alevins among and within redds are also linked to reproductive success and fitness (e.g., Hutchings 1991). The consistent increase in average size with later spawning efforts in our study is not explained by the measured environmental parameters, nor was it reported for chinook salmon (Field-Dodgson 1988). The size difference may be a function of genetic differences in embryo physiology (Balon 1980) suggested by varying colour forms of emerged alevins (Curry 1993). Later spawners may also produce larger eggs, leading to longer developmental periods (Rombough 1985) and larger alevins (Hutchings 1991). The declining size over time within individual redds was consistent with the experiments of Hausle and Cobble (1976), but suggests that later emergers are less fit given the feeding advantages related to size for young-of-the-year (Grant 1990). Elliott's (1989) hypothesis that a critical period determining survival occurs at first feeding also suggests that emergence of smaller alevins later is counterintuitive. Experiments are required to reconcile the influence of both environmental (e.g., temperature and oxygen) and biological (e.g., genetic control of embryo physiology and postemergence advantages) factors on the timing and size of alevins at emergence. Additional studies monitoring the genotypic composition of year-classes in several generations are necessary to determine the effect of these and other life-history tactics on the reproductive success of individuals and populations.

Acknowledgements

This project could not have been completed without the logistic and financial support of the Haliburton-Hastings and Algonquin Fisheries Assessment Units, Ontario Ministry of Natural Resources. Funding was also provided by a Department of Fisheries and Oceans science subvention grant and Natural Sciences and Engineering Research Council operating grant A6981 to D.L.G.N. Field assistance was provided by D. Haskins through the Environmental Youth Corp Programme, and C. Brady, R. Hawkins, F. Hicks, R. Irwin, and J. Inglis.

References

Balon, E.K. 1980. Early ontogeny of the brook charr, *Salvelinus* (Baione) *fontinalis*. *In* Charrs. Salmonid fishes of the genus *Salvelinus*. *Edited by* E.K. Balon. Dr. W. Junk Publishers, The Hague, Netherlands. pp. 631–666.

Bams, R.A. 1969. Adaptions of sockeye salmon associated with incubation in stream gravels. *In* Proceedings of the Symposium on Salmon and Trout in Streams. *Edited by* T.G. Northcote. University of British Columbia, Vancouver, B.C.

Benson, H.G. 1953. The importance of groundwater to trout populations in the Pigeon River, Michigan. J. North Am. Wildl. Conf. 18: 269–281.

Brannon, E.L. 1987. Mechanisms stabilizing salmonid fry emergence timing. *In* Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. *Edited by* H.D. Smith, L. Margolis, and C.C. Wood. Can. Spec. Publ. Fish. Aquat. Sci. No. 96.

Carline, R.F. 1980. Features of successful spawning site development for brook trout in Wisconsin ponds. Trans. Am. Fish. Soc. 109: 453–457.

Cunjak, R.A., and G. Power. 1986. Winter habitat utilization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. 43: 1970–1981.

Curry, R.A. 1993. Hydrogeology of brook charr (*Salvelinus fontinalis*) spawning and incubation habitats: linking aquatic and terrestrial habitats. Ph.D. thesis, University of Guelph, Guelph, Ont.

Curry, R.A., and D.L.G. Noakes. 1995. Groundwater and the selection of spawning sites by brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 52: 1733–1740.

Curry, R.A., P.M. Powles, J.M. Gunn, and V.A. Liimatainen. 1991. Emergence chronology of brook charr, *Salvelinus fontinalis*, alevins in an acidic stream. Environ. Biol. Fishes, 31: 25–31.

Curry, R.A., S. Allen, M.G. Fox, and G.E. Morgan. 1993. Growth and food of young-of-the-year brook charr, *Salvelinus fontinalis*, in lake and creek environments. Environ. Biol. Fishes, 37: 131–138.

Day, R.W., and G.P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr. 59: 433–463.

Dill, L.M., and T.G. Northcote. 1970. Effects of gravel size, egg depth and egg density on intra-gravel movement and emergence of coho salmon (*Oncorhynchus kisutch*) alevins. J. Fish. Res. Board Can. 27: 1191–1199.

Elliott, J.M. 1989. Mechanisms responsible for population regulation in young migratory trout, *Salmo trutta*. I. The critical time for survival. J. Anim. Ecol. 58: 987–1001.

Field-Dodgson, M.S. 1988. Size characteristics and diet of emergent chinook salmon in a small, stable, New Zealand stream. J. Fish Biol. 32: 27–40.

Fiss, F.C., and R.F. Carline. 1993. Survival of brook trout embryos in three episodically acidified streams. Trans. Am. Fish. Soc. 122: 268–278.

Franzin, W.G., and S.M. Harbicht. 1985. An evaluation of the relative success of naturalized brook charr, *Salvelinus fontinalis* (Mitchill), populations in South Duck River and Cowan Creek, Duck Mountain Region, Manitoba. Can. Tech. Rep. Fish. Aquat. Sci. No. 1370.

Fraser, J.M. 1985. Shoal spawning of brook trout, *Salvelinus fontinalis*, in a Precambrian Shield lake. Nat. Can. (Que.), 112: 163–174.

Freeze, R.A., and J.A. Cherry. 1979. Groundwater. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Gardiner, W.R., and P. Geddes. 1980. The influence of body composition on the survival of juvenile salmon. Hydrobiologia, 69: 67–72.

Garside, E.T. 1966. Effects of oxygen in relation to temperature on the development of embryos of brook trout and rainbow trout. J. Fish. Res. Board Can. 23: 1121–1134.

Grant, J.W.A. 1990. Aggressiveness and the foraging behaviour of young-of-the-year brook charr (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 47: 915–920.

Grant, J.W.A., and D.L.G. Noakes. 1987. Movers and stayers: foraging tactics of young-of-the-year brook charr, *Salvelinus fontinalis*. J. Anim. Ecol. 56: 1001–1013.

Gunn, J.M. 1986. Behaviour and ecology of salmonid fishes exposed to episodic pH depressions. Environ. Biol. Fishes, 17: 241–252.

Hausle, D.A., and D.W. Coble. 1976. Influence of sand in redds on survival and emergence of brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. 105: 57–63.

Hayes, P.R., I.R. Wilmot, and D.A. Livinstone. 1951. The oxygen consumption of the salmon egg in relation to development and activity. J. Exp. Zool. 377–395.

Heming, T.A. 1982. Effects of temperature on utilization of yolk by chinook salmon (*Oncorhynchus tshawytscha*) eggs and alevins. Can. J. Fish. Aquat. Sci. 39: 184–190.

Hokanson, K.E.F., J.H. McCormick, B.R. Jones, and J.H. Tucker. 1973. Thermal requirements for maturation, spawning, and embryo survival of the brook trout, *Salvelinus fontinalis*. J. Fish. Res. Board Can. 30: 975–984.

Hunt, R.L. 1969. Overwinter survival of wild fingerling brook trout in Lawrence Creek, Wisconsin. J. Fish. Res. Board Can. 26: 1473–1483.

Hutchings, J.A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout, *Salvelinus fontinalis*. Evolution, 45: 1162–1168.

Lecren, E.D. 1962. The efficiency of reproduction and recruitment in freshwater fish. *In* The exploitation of natural animal populations. *Edited by* E.D. LeCren and M.W. Holdgate. J. Wiley and Sons, Inc., New York. pp. 283–296.

Marten, P.S. 1992. Effects of temperature variation on the incubation and development of brook trout eggs. Prog. Fish-Cult. 54: 1–6.

Matthess, G. 1982. The properties of groundwater. J. Wiley and Sons, Inc., Toronto, Ont.

McCart, P., and H. Bain. 1974. An isolated population of Arctic char (*Salvelinus alpinus*) inhabiting a warm mineral spring above a waterfall at Cache Creek, Northwest Territories. J. Fish. Res. Board Can. 31: 1408–1414.

Needham, P.R., and A.C. Jones. 1959. Flow, temperature, solar radiation, and ice in relation to activities of fishes in Sagehen Creek, California. Ecology, 40: 465–474.

Noakes, D.L.G. 1989. Early life history and behaviour of charrs. Physiol. Ecol. Jpn. (Spec. Vol.), 1: 173–186.

Nunan, C.P., and D.L.G. Noakes. 1985. Response of rainbow trout (*Salmo gairdneri*) embryos to current flow in simulated substrates. Can. J. Zool. 63: 1813–1815.

Peterson, R.H., H.C.E. Spinney, and A. Sreedharan. 1977. Development of Atlantic salmon (*Salmo salar*) eggs and alevins under varied temperature regimes. J. Fish. Res. Board Can. 34: 31–43.

Rombough, P.J. 1985. Initial egg weight, time to maximum alevin wet weight, and optimal ponding times for chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 42: 287–291.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6. 4th ed. SAS Institute Inc., Cary, N.C.

Silver, S.J., C.E. Warren, and P. Doudoroff. 1963. Dissolved oxygen requirements of developing steelhead trout and chinook salmon embryos at different water velocities. Trans. Am. Fish. Soc. 92: 327–343.

Snucins, E.J., R.A. Curry, and J.M. Gunn. 1992. Brook trout (*Salvelinus fontinalis*) embryo habitat and timing of alevin emergence in a lake and stream. Can. J. Zool. 70: 423–427.

Sokal, R.R., and F.J. Rohlf. 1981. Biometry. 2nd ed. W.H. Freeman and Co., New York.

Sowden, T.K., and G. Power. 1985. Prediction of rainbow trout embryo survival in relation to groundwater seepage and particle size of spawning substrates. Trans. Am. Fish. Soc. 114: 804–812.

Wetzel, R.G. 1983. Limnology. 2nd ed. Saunders College Publishing, Toronto, Ont.

Witzel, L.D., and H.R. MacCrimmon. 1983. Embryo survival and emergence of brook charr, *Salvelinus fontinalis*, and brown trout, *Salmo trutta*, relative to redd gravel composition. Can. J. Zool. 61: 1783–1792.

Sulphate mass balances of Precambrian Shield wetlands: the influence of catchment hydrogeology

Kevin J. Devito

Abstract: The sulphate budgets of two valley-bottom conifer swamps, located in headwater catchments with contrasting till depth, typical of the southern Canadian Shield were determined from June 1990 to August 1992. Temporal variations in the retention of SO_4^{2-} in both swamps were related to the magnitude of upland runoff and water level in the wetlands. Contrasts in catchment till depth and nature of groundwater flow resulted in differences in SO_4^{2-} retention between years and swamps. Transient groundwater inputs entering the swamp in the thin-till catchment resulted in a large water-table drawdown during dry summers, export of SO_4^{2-} the following fall, and low annual SO_4^{2-} retention. Continuous discharge of groundwater into the swamp in the moderate-till catchment maintained saturated surfaces and efficient SO_4^{2-} retention during both years with dry and wet summers. Greater SO_4^{2-} retention in this swamp may also be contributed by the greater interaction of groundwater, rich in SO_4^{2-}, with deeper peat. Data from these two sites indicate that within the shallow till – rock ridge physiographic region of the Canadian Shield, small variations in catchment till depth can result in a large range in temporal and spatial variations in SO_4^{2-} retention in valley wetlands.

Résumé : De juin 1990 à août 1992, nous avons établi le bilan de sulfate de deux marécages à résineux formés au fond de vallées situées dans la partie d'amont de bassins versants caractéristiques du sud du Bouclier canadien et se distinguant par un till glaciaire d'épaisseur différente. Les variations temporelles de la rétention de SO_4^{2-} étaient liées au niveau de l'eau dans les marécages et au volume de ruissellement. La différence de l'épaisseur du till glaciaire du bassin hydrographique et le mode d'écoulement des eaux souterraines ont déterminé des différences de rétention d'une année à l'autre et d'un marécage à l'autre. L'intermittence des apports d'eau souterraine dans le marécage du bassin versant à till glaciaire de faible épaisseur a entraîné un important rabattement durant les étés secs, la sortie de SO_4^{2-} l'automne suivant et une faible rétention annuelle de SO_4^{2-}. Dans le marécage du bassin versant à till glaciaire d'épaisseur moyenne, les apports d'eau souterraine ont été continus, si bien que, tant durant les années à été sec que durant les années à été humide, il est resté des surfaces saturées et la rétention de SO_4^{2-} a été appréciable. Il se peut que la rétention supérieure dans ce marécage ait aussi été due à une interaction plus importante de l'eau souterraine, riche en SO_4^{2-}, avec la couche de tourbe, plus épaisse. Les données recueillies sur les deux marécages indiquent que dans la région physiographique à till peu épais et à crêtes rocheuses du Bouclier canadien, de faibles variations de l'épaisseur du till dans les bassins hydrographiques peuvent correspondre à de grandes plages de variation temporelle et spatiale de la rétention de SO_4^{2-} dans les marécages formés au fond des vallées.
[Traduit par la Rédaction]

Introduction

Input–output budgets, or examination of stream outflow chemistry, indicate that individual wetland sites may have high retention (Hemond 1980; Urban and Bayley 1986; Wieder 1985) or low retention (Brown 1985; Lundin and Bergquist 1990), or may alternate from low retention to net release (LaZerte 1993) of SO_4^{2-} on an annual basis. Seasonal mass balances have shown that SO_4^{2-} is generally retained in wetlands during the growing season when flow rates are low, whereas little retention and high SO_4^{2-} concentrations at the outlet occur during periods of high flow (Bayley et al. 1986; LaZerte 1993; Lundin and Bergquist 1990). Cyclic release of previously retained SO_4^{2-} has been observed in some wetlands following water-table drawdown and oxidation of reduced S during dry summers (Bayley et al. 1986; Wieder 1985). Although many studies have described the overall function of individual

Received August 31, 1994. Accepted March 3, 1995.
J12556

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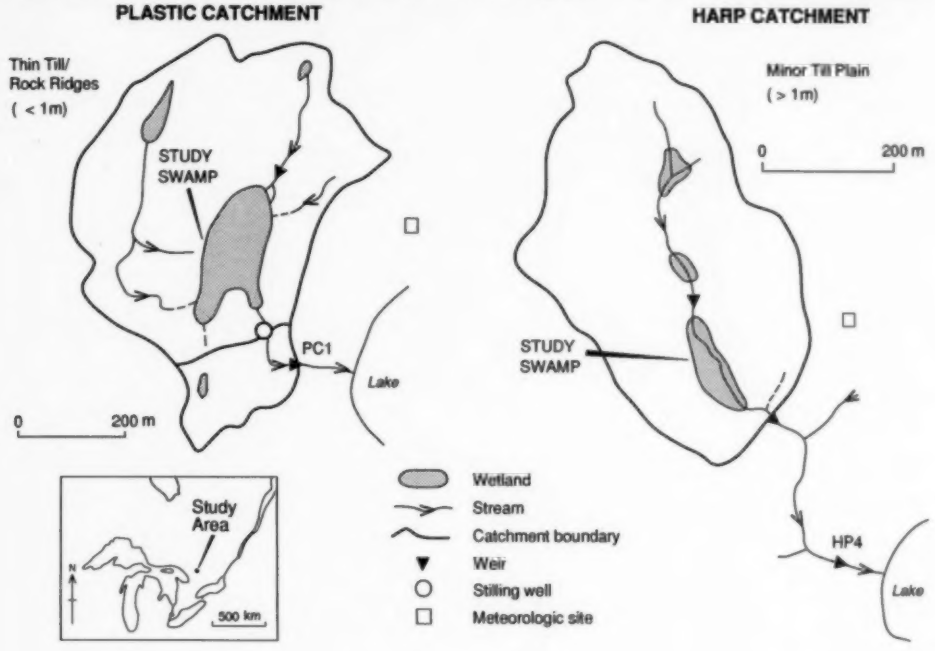
Fig. 1. Location of the study swamps and hydrometeorology stations in Plastic and Harp catchments.



wetlands, few studies have provided insight into the hydrogeologic processes that produce these observed variations in behaviour.

Water-table elevation, hydraulic gradient, and water retention capacity of the substrate largely control the magnitude, rate, and type of flow path (Hammer and Kadlec 1986) and sediment anoxia (Sparling 1966) and thus patterns of elemental transport and transformation within a wetland. The flow path of source water will also influence the degree and location of interaction with the wetland (Hill 1990; Phillips et al. 1993). Consequently, both external and internal hydrologic pathways will determine whether a wetland acts as an element sink or source. Thus, wetland biogeochemistry must be examined in the context of catchment hydrogeology to achieve generalizations that would facilitate the extension of results from the study of one wetland to others.

To determine the variability of SO_4^{2-} retention in wetlands, mass balances were determined for two valley-bottom forested swamps located in headwater catchments of the Canadian Shield. The two study sites are in catchments that represent the ends of the physiographic continuum, characteristic of the shallow till – rock ridge physiographic regions of the southern Canadian Shield, with till depths of <1 m and up to 10 m depth, respectively. In concurrent studies Devito et al. (1995) found that the valley-bottom wetlands in these two headwater catchments had a range in upland–wetland groundwater linkage and different seasonal patterns of surface saturation and water levels. In comparing the SO_4^{2-} mass balances of sites in catchments with contrasting till depth I ask how differences in upland–wetland linkages, as controlled by hydrologic setting, influence variations in SO_4^{2-} retention in valley-bottom forested swamps, the dominant wetland type in this landscape (Riley 1988). Specifically, with an increase in till depth, (*i*) does the SO_4^{2-} chemistry of different upland hydrologic sources influence patterns of SO_4^{2-} retention, and (*ii*) do variations in upland water source and wetland surface saturation result in different patterns of SO_4^{2-} retention?

Study site

The two wetland catchments are in headwater streams of Harp Lake (45°23′N, 79°08′W) and Plastic Lake (45°11′N, 78°50′W) located near the southern limit of the Precambrian Shield in south-central Ontario (Fig. 1). Annual precipitation in the area is 900–1100 mm with 240–300 mm falling as snow between December and April. The mean January and July air temperatures are −10 and 17.7°C, respectively. Annual runoff is similar in the two catchments, varying from 400 to 600 mm. The physiography, geology, hydrology, and geochemistry of Harp and Plastic Lake catchments have been reported in Devito et al. (1995), Devito and Dillon (1993), Hinton et al. (1993), and Renzetti et al. (1992).

Plastic conifer swamp (2.2 ha) occupies a central bedrock depression that represents about 10% of the 21.1-ha catchment (Fig. 1). The swamp is forested with white cedar (*Thuja occidentalis*) and black spruce (*Picea mariana*) with some birch (*Betula*) and maple (*Acer*). There is an understorey of *Alnus* spp. and *Ilex verticillata* and a well-developed layer of *Sphagnum*. Peaty, humic mesisols up to 6 m depth (average 2–3 m) overlie regions of gyttja

and deposits of silt, clay, sands and gravel up to 1 m depth in the bedrock basin.

Harp conifer swamp (1.2 ha) occupies the valley bottom and represents 5% of the 22.7-ha catchment (Fig. 1). The swamp is dominated by white cedar with considerable birch and maple. It has a poorly developed shrub and bryophyte mat. The average swamp soil depth is 2–3 m of peaty, cumula humisols, with deposits in excess of 5 m at two locations. The peat overlies layers (to 1 m) of gyttja and pockets of silt, clay, sand, and gravel.

Both catchments are underlain by impermeable Precambrian metamorphic silicate bedrock covered with thin basal till. Owing to the shallow till and hilly terrain only local groundwater aquifers develop and the hydrology varies seasonally (Devito et al. 1995). Overburden in Plastic catchment is classified as thin till – rock ridges with depths <1 m over most of the catchment. Both stream and groundwater flow within the catchment are transient, running dry for up to several months during the summer and several weeks during the winter low-flow period (Devito et al. 1995). Upland–swamp interactions are near the peat surface in Plastic swamp as groundwater from shallow soil and sandy basal till and stream inputs discharge along the swamp margin.

In most of the catchment above the Harp swamp the surficial geology is classified as minor till (>1 m depth), with deposits of sand on the north side of the stream representing about 29% of the catchment area. Owing to the increase in till depth there are several flow paths of water connecting the swamp to the uplands (Devito et al. 1995). Stream inputs and groundwater from shallow soil and till interact primarily at the swamp–upland contact. In contrast to Plastic swamp, Harp swamp is situated in a groundwater discharge zone. Slow-moving groundwater discharges from beneath the peat throughout the year.

Methods

Hydrometeorology

Meteorologic data for estimating evapotranspiration (a modified Penman–Monteith combination model), precipitation, and atmospheric deposition were collected at meteorologic stations located within 1 km of each swamp (Fig. 1) as described by Devito et al. (1995). The total change in water storage was determined from the change in water volume in the peat and the accumulation of snow in the swamp. The water table was continuously monitored throughout the study and the specific yield of the peat was estimated from the bulk density of 0–50 cm peat as described by Boelter and Verry (1977). Snow cores were collected at two locations in each swamp at the beginning of the month in January to April in 1991 and at the end of March 1992 to estimate water volume stored in the snow pack.

Stream stages at the catchment outflow and the outflow and main inflow of both swamps were continuously monitored at 90° V-notch weirs (Fig. 1). When stage records were not available (e.g., because of ice), discharge was estimated by regressing mean daily discharge (MDD) with data from a nearby weir (Fig. 1). Instantaneous discharge at two inflow streams to Plastic swamp was measured on an event basis. Mean daily discharge was calculated either by linear integration of instantaneous discharge measurements or by regression with MDD at the adjacent weir (see Devito and Dillon 1993).

Groundwater wells and piezometers were installed along longitudinal and transverse transects in both swamps as described in Devito et al. (1995). Water-table level and piezometric head in both swamps were monitored at least once a month at all locations, often more frequently, but only twice from December to March owing to problems with ice. Groundwater wells in the hillslope and swamp perimeter were used to estimate hillslope runoff on an event basis or at least once a week during lower flows. Water-level elevation in one hillslope well was continuously monitored, allowing for daily estimate of hillslope runoff from that location. Water-table gradients at other hillslope wells were occasionally supplemented by regression with the continuously monitored well. The hydraulic conductivity of selected piezometer nests and all groundwater wells in the hillslopes was determined by the Hvorslev water recovery method (Freeze and Cherry 1979).

Shallow groundwater flow entering Plastic swamp was estimated by prorating unit area runoff for the entire area above the swamp, as calculated from the gauged inflow stream (Fig. 1) and subtracting the measured stream discharge (Devito and Dillon 1993). In hydrologic studies lined runoff plots within the Plastic catchment estimated the unit runoff at the gauged inflow stream very well, justifying the use of this stream to estimate the unit runoff for the entire hill slope surrounding the swamp (Renzetti et al. 1992).

Groundwater flow entering Harp swamp was calculated using Darcy's Law:

$$Q = K_{sat} \cdot A \cdot \mathrm{d}h/\mathrm{d}l$$

where Q is discharge (L/s), K_{sat} is the saturated hydraulic conductivity (cm/s), A is the cross-sectional area of flow (cm^2), and $\mathrm{d}h/\mathrm{d}l$ is the hydraulic gradient.

The total groundwater flow was determined assuming that flow nets from the hillslopes can be separated from groundwater discharging down the valley and upwelling beneath the peat. The two groundwater sources are referred to as hillslope groundwater and groundwater upwelling from below the peat, respectively.

In Harp catchment, hillslope groundwater was divided into shallow groundwater moving through soils (<0.5 m) of higher K_{sat} (10^{-2}–10^{-3} cm/s) and deep groundwater moving through glacial tills of lower K_{sat} (<10^{-4} cm/s). Dupuit–Forchheimer flow was assumed for shallow hillslope groundwater moving through the top 0.5 m of soil (Freeze and Cherry 1979). Hydraulic gradient was estimated from the series of wells at the hillslope and swamp perimeter. The depth of saturated soil above the less permeable layer (generally at 0.5 m depth) was used to determine the cross-sectional area (A). Deeper hillslope groundwater was estimated using the average K_{sat} and flow nets produced from the piezometer nest on the hillslope and swamp perimeter. A was determined on the basis of the maximum depth to which the piezometers at the hillslope nests could be driven (range 1–2.5 m).

Groundwater upwelling below the peat was determined by the vertical hydraulic head differences between the

piezometer below or near the peat–mineral soil interface and the next shallowest piezometer in nests along the longitudinal transect. The seepage area (A) around each nest was estimated as a polygon with the piezometer nest being the centre, the length extending from piezometer nests at the wetland perimeters, and the width extending half the distance to the piezometer nest up slope and down slope along the longitudinal transect.

Water chemistry

Sulphate concentrations of stream and water in shallow soils were sampled at least once a week, with an increase in frequency of one to several times daily during peak flows. Groundwater from shallow soils and tills was sampled from a series of shallow wells buried in mineral substrate at the foot of the hillslopes. Sulphate concentrations of groundwater from deeper tills in the hillslopes adjacent to Harp swamp were determined on six dates during 1991–1992 from water sampled from piezometers, at 1 m depth and greater, located at the foot of the hillslope and wetland margin. Groundwater upwelling beneath the peat in Harp swamp was sampled from piezometers extending into the underlying mineral substrate at piezometer nests located along a transect longitudinal to stream flow down the valley.

Water for SO_4^{2-} analysis was refrigerated until analyzed, usually within 48 h of sampling. Sulphate was determined by ion chromatography (Ontario Ministry of the Environment 1984).

Sulphate budgets

Sulphate load was estimated by combining continuous daily measurements of groundwater and stream runoff with instantaneous estimates of SO_4^{2-} concentrations as outlined in Devito and Dillon (1993). When more than one SO_4^{2-} sample was collected at a stream, well, or piezometer the average of the samples for that day was used as an instantaneous estimate. Atmospheric deposition was determined from the volume and the SO_4^{2-} concentration of water collected for each sampling period (Dillon et al. 1988).

In determining the SO_4^{2-} load of groundwater from shallow soils the hillslopes were divided along the major axis of the swamps. There were five and six sets of groundwater wells on the west-north slope and east slope of Plastic swamp, respectively. In Harp, four sets of groundwater wells on the north-west slope and only one set of wells from the south slope were used. The average SO_4^{2-} concentrations from the hillslope wells were used for a given sampling.

In Harp swamp, sulphate concentrations from shallow groundwater wells and deeper groundwater were similar on six dates sampled in 1991–1992. The average daily SO_4^{2-} concentrations of deeper groundwater were assumed to be the same as concentrations in shallow groundwater wells. Samples from the six dates in 1991–1992 suggest that spatial variation is greater than seasonal variation in the SO_4^{2-} concentrations of upwelling groundwater beneath the peat in Harp swamp. The average SO_4^{2-} concentration from different sampling dates and various piezometers in the underlying mineral substrate was multiplied by the volume of water upwelling at each piezometer nest location. The SO_4^{2-} concentrations of upwelling groundwater in 1990-1991 were assumed to be the same as in 1991–1992. The SO_4^{2-} concentrations of groundwater moving below the outlet weir in Harp swamp were assumed to be the same as those of water in deep piezometers at the three sites nearest the swamp outlet.

All discharge estimates, converted to swamp runoff (mm, equivalent to $L \cdot m^2$), and loads (g $SO_4^{2-} \cdot m^2$) for each time period were summed into summer (June to August), fall (September to November), winter (December to February), and spring (March to May) seasons. The hydrologic year was from June 1 to May 31.

Error estimates

The uncertainty associated with the water budgets was estimated from minimum and maximum estimates of budget components. Estimating the budget variance by least-square errors is preferable (e.g., Devito and Dillon 1993; Winter 1981); however, minimum and maximum estimates were the only measure of variability that could be readily assessed, in this study, because of low sample size for many hydrologic parameters. The minimum and maximum SO_4^{2-} concentrations of samples used to determine average daily concentrations were multiplied by the minimum and maximum estimates, respectively, of daily water volumes for groundwater and stream runoff. The analytical error for the various budget components ranged from 8 to 11% for SO_4^{2-} and was generally less than the spatial and temporal variability. The minimum and maximum SO_4^{2-} load was estimated for each component in the budget and summed to determine the uncertainty for seasonal and annual estimates.

Minimum and maximum MDD at gauged weirs were estimated from the stage–discharge curve by calculating the discharge with the appropriate plus–minus error of stage estimates and added for monthly volumes. In translating potentials (in millivolts) or strip charts, stream stage could be determined with an accuracy of ±0.002 m for most gauged weirs, but ±0.005 m at Plastic swamp outlet owing to wave action in the pool.

Errors associated with estimating discharge or groundwater flow by regression with a gauged weir were calculated either as (Herschey 1973)

$$SD = (\Sigma(\text{obs} - \text{pred})^2/(n - 1))^{0.5}$$

or as

$$\%ERR = (\Sigma(((\text{obs} - \text{pred})/\text{obs}) \cdot 100)^2/(n - 1))^{0.5}$$

SD or %ERR was used depending on which measure was more homogeneous over the range of discharge measurements.

The range in estimating groundwater runoff from the hillslope was determined from minimum and maximum head measurements in the different piezometer nests or combination of the series of wells used for determining hydraulic gradient. Minimum and maximum volumes of upwelling groundwater from beneath the swamp were determined from the range in K_{sat}, as multiple head measures were not available.

The ranges of average snow depth and water equivalent at the two locations in each swamp were used. The

Table 1. Water budget (mm) for Plastic swamp, 1990–1991 and 1991–1992.

	Stream inflow	Shallow groundwater	Precipitation	Total inputs	ET	Stream outflow	Total outputs	Total storage change	Balance	
									Absolute	% of inputs
Summer 1990	3	14	120	137	325	22	347	−106	−105	−77
Fall 1990	538	1046	373	1957	101	1617	1718	205	34	2
Winter 1991	120	403	224	747	0	713	713	81	−48	−6
Spring 1991	1197	2094	309	3600	116	4307	4423	−131	−692	−19
Annual (1991–1992)	1859	3557	1025	6440	542	6659	7201	50	−810	−13
Half range	637	612	205	1454		1814	1814	8		
%[a]	29	55	16		8	92				
Summer 1991	9	40	219	267	358	109	466	−96	−104	−39
Fall 1991	212	624	300	1135	108	1080	1188	116	−169	−15
Winter 1992	159	425	230	814	0	827	827	103	−115	−14
Spring 1992	728	1583	181	2492	110	2118	2228	−146	410	16
Annual (1991–1992)	1107	2671	930	4708	576	4134	4710	−24	22	0
Half range	348	520	186	1055		1234	1234	9		
%[a]	24	57	20		12	88				
Summer 1992	37	144	274	455	350	204	554	54	−152	−33

Note: Shown are annual means and half the range (maximum − minimum) for each component. ET, evapotranspiration.
[a]Relative to total inputs and outputs.

error associated with measuring precipitation depth was previously estimated to be ±20% per month (Devito and Dillon 1993) and assumed to be ±20 % for SO_4^{2-} load. Errors associated with estimating evapotranspiration can be substantial (Winter 1981) but are unknown for the Penman–Monteith model and were not determined.

Results

Water table fluctuations

There were differences between Plastic swamp and Harp swamp in water level fluctuations during the summer as a consequence of the transient and continuous nature of groundwater flow in the two catchments, respectively (Fig. 2). Rainfall in June–August of 1990 to 1992 was 120, 219, and 274 mm in Plastic swamp and 160, 191, and 213 mm in Harp swamp, respectively (Tables 1 and 2). The water table in Plastic swamp dropped to about 60 cm below the surface in the summer of 1990, whereas in the summer of 1991 and 1992 it declined by only about 20 and 10 cm, respectively. In contrast to the findings in Plastic swamp, groundwater and stream inputs to Harp swamp occurred continuously and the water table remained relatively stable, within 10 cm of the peat surface during the three summers of study.

The period and amplitude of water level relative to the surface in both Plastic and Harp swamps varied seasonally (Fig. 2). The water table was above the peat surface during the late fall, early winter, and spring when runoff inputs from the surrounding catchment were high. Peaks in water level were associated with rain and snowmelt events, with the greatest water levels during spring snowmelt in March and April. Water levels remained near or below the peat during the summer and for a short time in the winter when runoff inputs were small.

Water balance

The inputs of water balanced the outputs, within the range of estimates for total inputs and outputs, in both swamps for both 1990–1991 and 1991–1992 (Tables 1 and 2). The water budget for Plastic swamp closed within 13 and 0%, and for Harp swamp within 12 and 17%, of the inputs for 1990–1991 and 1991–1992, respectively.

Seasonally, more than 50% of the inputs and outputs occurred during spring and less than 10% during the summer in Plastic swamp (Table 1). During the summer, stream and groundwater contributions were low (<20%) while precipitation contributed >80 % of the total summer inputs. Evapotranspiration represented >90 and 75% of the summer outputs in 1990 and 1991, respectively. In Harp swamp more than 40% of the inputs and outputs occurred during spring, and about 10–15% during the summer (Table 2). During the summer the precipitation contribution remained low, about 10%, and shallow and deep groundwater represented 25–35 and 25–30%, respectively, of the total summer inputs. The inflow stream contributed 30–35% of the summer inputs and evapotranspiration represented as much as 33% of the summer outputs in Harp swamp.

Table 2. Water budget (mm) for Harp swamp, 1990–1991 and 1991–1992.

		Hillslope										Balance	
	Stream inflow	Shallow groundwater	Deeper groundwater	Upwelling groundwater	Precipitation	Total inputs	Groundwater	ET	Stream outflow	Total outputs	Total storage change	Absolute	% of inputs
Summer 1990	648	736	211	352	160	2 107	97	324	971	1 392	−40	754	36
Fall 1990	1736	700	267	280	372	3 355	96	92	2 664	2 852	92	411	12
Winter 1991	1405	987	306	349	222	3 268	65	0	2 220	2 315	85	868	27
Spring 1991	4164	1115	341	1136	279	7 035	97	163	6 953	7 213	−114	−64	−1
Annual (1990–1991)	7953	3537	1124	2117	1033	15 764	384	579	12 808	13 771	23	1970	12
Half range	1342	510	290	728	207	3 078	352	—	1 534	1 886	4		
%[a]	50	22	7	13	7		3	4	93				
Summer 1991	511	452	174	271	191	1 596	97	357	630	1 083	−32	546	34
Fall 1991	1323	506	248	492	329	2 899	96	94	2 185	2 375	64	460	16
Winter 1992	1358	841	285	589	219	3 294	96	0	2 214	2 310	111	873	27
Spring 1992	3242	964	328	444	175	5 153	97	165	4 730	4 992	−140	301	6
Annual (1991–1992)	6434	2763	1035	1797	914	12 943	385	616	9 759	10 760	3	2181	17
Half range	666	633	284	278	183	2 044	353	—	725	1 078	1		
%[a]	50	21	8	14	7		4	6	91				
Summer 1992	807	423	153	564	213	2 160	97	352	1 166	1 615	6	540	25

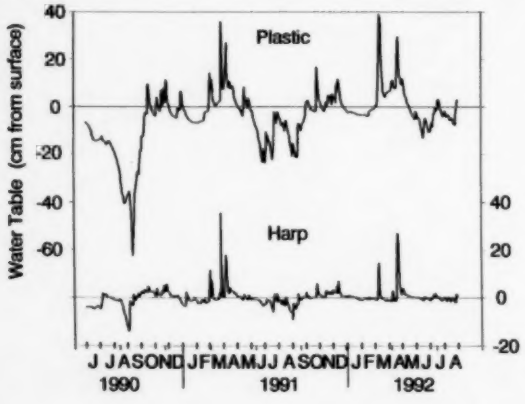
Note: Shown are annual means and half the range (maximum − minimum) for each component. ET, evapotranspiration.
[a]Relative to total inputs and outputs.

Table 3. Sulphate budget (g/m^2) for Plastic swamp, 1990–1991 and 1991–1992.

	Stream inflows	Hillslope groundwater	Atmospheric deposition	Total inputs	Total outputs (Stream outflow)	Balance in–out	
						Absolute	% of inputs
Summer 1990	0.0	0.1	0.5	0.6	0.1	0.5	84
Fall 1990	4.8	9.9	1.0	15.7	19.4	−3.8	−24
Winter 1991	1.1	3.6	0.3	5.0	6.8	−1.8	−36
Spring 1991	8.7	16.7	0.7	26.1	29.6	−3.5	−14
Annual (1990–1991)	14.5	30.4	2.5	47.4	56.0	−8.6	−18
Half range	5.0	5.2	0.3	10.1	16.0		
%[a]	31	64	5				
Summer 1991	0.1	0.3	0.7	1.1	1.2	−0.1	−12
Fall 1991	1.7	5.7	0.7	8.1	10.1	−2.0	−24
Winter 1992	1.3	3.9	0.4	5.6	6.6	−0.9	−17
Spring 1992	5.6	13.7	0.5	19.8	15.3	4.4	22
Annual (1991–1992)	8.7	23.6	2.3	34.6	33.2	1.4	4
Half range	2.7	4.6	0.2	7.3	10.0		
%[a]	25	68	7				
Summer 1992	0.3	1.0	0.8	2.1	0.7	1.4	67

Note: Shown are annual means and half the range (maximum − minimum) for each component.
[a]Relative to total inputs and outputs.

Fig. 2. Water table relative to the peat surface in the central portion of Plastic and Harp swamps, June 1990 to August 1992.



Sulphate budgets

The relative contributions of the various inputs to Plastic and Harp swamps were similar for the 2 years (Tables 3 and 4). On an annual basis, hillslope groundwater was the dominant source of SO_4^{2-} (64–68%) to Plastic swamp, followed by stream inputs (25–31%) and atmospheric deposition (5–7%) (Table 3). Similarly, groundwater was the dominant source of SO_4^{2-} (57%) to Harp swamp, followed by stream inputs (40%) (Table 4). Upwelling groundwater from beneath the peat represented about 21% of the total annual SO_4^{2-} inputs to Harp swamp. Atmospheric deposition inputs to Harp swamp were minor (2%).

The trends in seasonal variations of total inputs to the two swamps were similar, although the magnitude of this variation was not as large in Harp swamp. Summer and winter inputs represented <5 and 11–16% of the annual inputs to Plastic swamp, respectively (Table 3). Fall and spring inputs represented 24–33 and 55–57% of the annual inputs, respectively. For Harp swamp 12% of the annual inputs occurred during the summer, about 20–30% each during the fall and winter, and 37–44% during the spring (Table 4).

The annual SO_4^{2-} retention differed between the two swamps. The differences between the inputs and outputs of SO_4^{2-} in Plastic swamp were within the budget uncertainties for 1990–1991 and 1991–1992. In 1990–1991 there was a weak export of SO_4^{2-} (−18%), which was greater than the −13% bias in the water balance. In contrast, the inputs of SO_4^{2-} exceeded the outputs in Harp swamp and indicated that SO_4^{2-} was retained during both years (Table 4). The retention, relative to the inputs, was 15–20% greater than the positive biases in the water budget in 1990–1991 and 1991–1992.

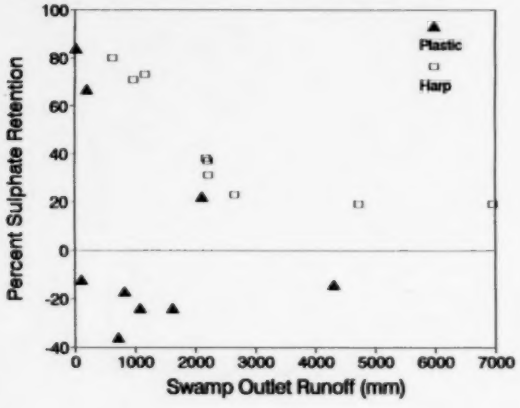
Seasonal variations in SO_4^{2-} retention appear to be associated with swamp hydrology. Estimates of SO_4^{2-} outputs exceeded the inputs during the fall of 1990 and 1991 in Plastic swamp, indicating a net export of SO_4^{2-} following water-table drawdown (Table 3). Inputs of SO_4^{2-} were similar to outputs during the summer of 1991, possibly

Table 4. Sulphate budget (g/m^2) for Harp swamp, 1990–1991 and 1991–1992.

		Hillslope								Balance	
	Stream inflow	Shallow groundwater	Deeper groundwater	Upwelling groundwater	Atmospheric deposition	Total inputs	Groundwater	Stream outflow	Total outputs	Absolute	% of inputs
Summer 1990	3.9	7.5	1.6	5.4	0.6	18.9	1.1	4.4	5.5	13.4	71
Fall 1990	14.7	9.3	2.7	4.3	1.0	32.0	1.1	23.6	24.7	7.3	23
Winter 1991	13.0	12.9	3.3	5.4	0.3	34.9	1.1	23.0	24.0	10.9	31
Spring 1991	31.0	13.6	3.4	17.9	0.6	66.5	1.1	52.5	53.6	12.9	19
Annual (1990–1991)	62.6	43.2	10.9	33.1	2.5	152.4	4.4	103.5	107.9	44.5	29
Half range	8.1	8.3	3.7	14.8	0.5	34.9	4.1	9.7	13.8		
%[a]	41	28	7	22	2		4	96			
Summer 1991	2.9	6.3	2.0	4.4	0.7	16.2	1.1	2.1	3.2	13.0	80
Fall 1991	10.4	7.3	2.6	7.4	0.8	28.4	1.1	16.5	17.6	10.8	38
Winter 1992	12.9	11.3	3.0	8.9	0.3	36.5	1.1	21.9	22.9	13.6	37
Spring 1992	25.5	12.1	3.2	6.7	0.5	48.0	1.1	37.6	38.7	9.3	19
Annual (1991–1992)	51.6	37.0	10.8	27.4	2.5	129.2	4.4	78.1	82.5	46.7	36
Half range	5.5	9.9	3.8	7.5	0.5	26.6	4.1	5.7	9.8		
%[a]	40	29	8	21	2		5	95			
Summer 1992	4.9	5.5	1.6	8.5	0.8	21.2	1.1	4.6	5.7	15.4	73

Note: Shown are annual means and half the range (maximum − minimum) for each component.
[a]Relative to total inputs and outputs.

Fig. 3. Seasonal percent sulphate retention relative to swamp outlet runoff (mm) in Plastic and Harp swamps, June 1990 to August 1992. A positive value indicates that sulphate is retained in the swamp (total inputs > total outputs) and a negative value indicates that sulphate is exported (total inputs < total outputs).



because of a large export of SO_4^{2-} during the midsummer rainstorms (K.J. Devito and A.R. Hill, unpublished data). Sulphate inputs exceeded outputs to Plastic swamp during the summers of 1990 and 1992, indicating efficient retention. In Harp swamp, inputs exceeded outputs in the summer and fall, indicating that SO_4^{2-} was retained during both seasons (Table 4).

Sulphate retention efficiency generally decreased with an increase in the magnitude of runoff in both swamps (Fig. 3). Although SO_4^{2-} was retained through the year in Harp swamp the percent retention was greatest during the summer and retention efficiency decreased with increase in runoff from fall and winter to spring (Table 4). The pattern was not as strong in Plastic swamp. There is a general trend of net retention of SO_4^{2-} during the summer and export during the fall and spring (Fig. 3). In contrast to Harp swamp, however, inputs exceeded estimates of outputs during several seasons following periods of large water-table drawdown and aeration of the wetland peat.

Discussion

Comparison of Plastic and Harp swamps, which are located in catchments with contrasting till depth, illustrates how critical the pattern of groundwater flow and water-table fluctuations can be in influencing SO_4^{2-} transformations and long-term retention among wetlands. Transient groundwater inputs and a large drawdown of the water table in Plastic swamp were associated with large net S mineralization in surface peat during the dry summer (K.J. Devito and A.R. Hill, unpublished data). An export of SO_4^{2-} during the fall and on an annual basis (−18%) was measured in Plastic swamp in 1990–1991. Mass balance studies have also measured a net annual export of SO_4^{2-} (−8 to −32%) from Plastic swamp in 4 years with dry summers during a 7-year period prior to this study (LaZerte 1993). In contrast, outputs roughly balanced inputs (+4%) in 1991–1992, a year with a moderate water-table drawdown during the summer. LaZerte (1993) measured net SO_4^{2-} retention (20–29%) in Plastic swamp during years with normal or greater summer rainfall. The mass balances reported by LaZerte (1993) and herein show that retention can vary by more than 40% with year to year differences in summer precipitation. The long-term net SO_4^{2-} retention in systems such as Plastic swamp is probably low owing to the swamp alternating between net retention and export.

In contrast, continuous inputs from deeper till maintained saturated conditions in Harp swamp during dry summers and SO_4^{2-} was retained more efficiently (29–36%) in both years of the study. The magnitude of the differences between retention in Plastic and Harp swamps appears to be greater than the errors in estimating the mass balances (the present study; Lazerte 1993). This suggests that the long-term SO_4^{2-} retention efficiency of wetlands not susceptible to large water-table drawdown will be greater than in wetlands susceptible to periodic drawdown of the water table. The mass of total S in Harp swamp peat (0 to 50 cm depth) is almost 2 times that in Plastic swamp (K.J. Devito and A.R. Hill, unpublished data). Whether the higher S content in Harp swamp is due to inhibition of S reoxidation and release during dry periods, increased proportion of catchment runoff, or greater interaction of groundwater with anoxic peat than in Plastic swamp is uncertain.

Small variations in till depth (1–2 m) and deeper sources of groundwater in headwater catchments potentially increase the SO_4^{2-} retention efficiencies in valley wetlands of the southern Shield landscape. The annual input from deeper groundwater, rich in SO_4^{2-}, represents a large portion of the net annual retention in Harp swamp (Table 4). Quantification and comparison of deeper groundwater inputs, however, are tenuous because of the relatively small inputs and errors in measuring groundwater flow. Nevertheless, a larger portion of the groundwater entering Harp came into direct contact with deeper peats (K.J. Devito and A.R. Hill, unpublished data).

Annual retention efficiency of SO_4^{2-} in Plastic and Harp swamps is lower than in a forested poor fen in northwest Ontario (Bayley et al. 1986) and forested bogs in Massachusetts (Hemond 1980) and Minnesota (Urban et al. 1989). Conversely, very low SO_4^{2-} retention efficiencies have been measured in some Swedish bogs (Lundin and Bergquist 1990) and a minerotrophic mire in England (Brown 1985). The retention efficiency declines as the proportion of the catchment contributing runoff to the wetland increases because of a disproportionately larger increase in the input of SO_4^{2-} relative to wetland area. However, the absolute rate of SO_4^{2-} uptake and the mass of S in peat increase with greater upland inputs of SO_4^{2-}. The S content of peat is considerably larger in Harp and, to a lesser extent, in Plastic than that measured in bogs which receive proportionately less upland inputs (K.J. Devito and A.R. Hill, unpublished data).

Part of the low annual retention in the study swamps results from decreased retention efficiency or net export in seasons with a large magnitude of upland runoff and SO_4^{2-} inputs. This has also been shown to control long-term P and N retention in wetlands in the study area (Devito

and Dillon 1993). Relationships between seasonal SO_4^{2-} retention and runoff magnitude have been observed in wetlands in other geographic locations (Lundin and Bergquist 1990; Urban et al. 1989). In the climate of central Ontario the majority of the annual flux of SO_4^{2-} to the valley wetlands occurs when vegetation is largely dormant, surface pathways dominate, and temperatures and aerobic conditions at the surface limit SO_4^{2-} uptake, resulting in low annual retention efficiencies. In temperate and boreal climates, small valley wetlands receiving a large proportion of catchment runoff may experience hydrologic conditions that maximize contact of inflowing water with vegetation and anoxic sediments, resulting in efficient SO_4^{2-} retention for only a few months during late spring and summer.

Conclusion

In comparing two wetland sites located in catchments that represent the ends of the continuum of local physiography (till depth) and hydrology (Devito et al. 1995) this study documents a large proportion of the range of SO_4^{2-} retention of wetlands in this landscape. Thus, the results from this research may be generalized to other wetlands located in various hydrogeologic settings within this particular landscape as well as other wetlands connected only to local aquifers.

Comparison of SO_4^{2-} mass balances in Harp and Plastic swamps shed light on the variability in S retention of wetlands within the Shield landscape in response to dry periods. Although many studies have suggested that drought and water-table drawdown are important to peaks in SO_4^{2-} concentrations in outlet streams and to net export from wetlands, the present study is the first to relate SO_4^{2-} export to wetland water table as controlled by catchment hydrogeology. Within headwater catchments of the Canadian Shield, small differences in till depth (1–2 m) between catchments are critical in determining the threshold response from ephemeral to continuous groundwater inputs. These differences in catchment hydrology can result in spatial and temporal variation in the SO_4^{2-} retention efficiency of various wetlands in the landscape.

Both study sites are classified as conifer swamps. However, their large variation in hydrology and S dynamics suggests that traditional wetland classifications, based largely on vegetation communities (National Wetlands Working Group 1988, e.g., bog, fen, swamp, and marsh), are of limited value in extrapolating studies of SO_4^{2-} retention from one wetland to others. To develop valid generalizations about wetland biogeochemistry an understanding of the wetland hydrogeologic setting is also required.

Acknowledgements

I thank David Cruickshank and Stan Sutey for assistance both in the field and the laboratory. I acknowledge the cooperation and logistical support provided by the staff at the Dorset Research Centre, in particular S. Barnes for laboratory assistance. I also thank C. Randall, York University, for drawing some of the graphs. I thank A. Hill, N. Roulet, and P. Dillon and two anonymous referees for helpful discussion and review of the manuscript. The work for this study was conducted while I was supported by a Natural Sciences and Engineering Research Council studentship and an Ontario Graduate Scholarship, and the research was funded by an Ontario Ministry of the Environment research grant.

References

Bayley, S.E., R.S. Behr, and C.A. Kelley. 1986. Retention and release of S from a freshwater wetland. Water Air Soil Pollut. 31: 101–114.

Boelter, D.H., and E.E. Verry. 1977. Peatland and water in the northern Lake States. U.S. For. Serv. Gen. Tech. Rep. No. NC-31.

Brown, K.A. 1985. Sulphur distribution and metabolism in waterlogged peat. Soil Biol. Biochem. 17: 39–45.

Devito, K.J., and P.J. Dillon. 1993. The influence of hydrologic condition and peat oxia on the phosphorous and nitrogen dynamics of a conifer swamp. Water Resour. Res. 29: 2675–2685.

Devito, K.J., N.T. Roulet, and A.R. Hill. 1995. Groundwater – surface water interactions in headwater forested wetlands of the Canadian Shield. J. Hydrol. (Amsterdam). In press.

Dillon, P.J, M. Lusis, R. Reid, and D. Yap. 1988. Ten-year trends in sulphate, nitrate and hydrogen deposition in central Ontario. Atmos. Environ. 22: 901–905.

Freeze, R.A., and J.A. Cherry. 1979. Groundwater. Prentice-Hall, Englewood Cliffs, N.J.

Hammer, D.E., and R.H. Kadlec. 1986. A model for wetland surface water dynamics. Water Resour. Res. 22: 1951–1958.

Hemond, H.F. 1980. Biogeochemistry of Thoreau's Bog, Concord, Massachusetts. Ecol. Monogr. 50: 507–526.

Herschey, R.W. 1973. The magnitude of errors at flow measurement stations. *In* Hydrometry: Proceedings of the Koblenz Symposium, 1970. Vol. 1. Unesco, World Meteorologic Organization, International Association of Hydrological Science. pp. 109-131.

Hill, A.R. 1990. Ground water flow paths in relation to nitrogen chemistry in the near-stream zone. Hydrobiologia, 206: 39–52.

Hinton, M.J., S.L. Schiff, and M.C. English. 1993. Physical properties governing groundwater flow in a glacial till catchment. J. Hydrol. (Amsterdam), 142: 229–249.

LaZerte, B.D. 1993. The impact of drought and acidification on the chemical exports from a minerotrophic conifer swamp. Biogeochemistry, 18: 153–175.

Lundin, L., and B. Bergquist. 1990. Effects on water chemistry after drainage of a bog for forestry. Hydrobiologia, 196: 167–181.

National Wetlands Working Group. 1988. Wetlands of Canada. Ecological Land Classification Series, No. 24. Environment Canada, Ottawa, Ont., and Polyscience Publications Incorporated, Montreal, Que.

Ontario Ministry of the Environment. 1984. Outlines of analytical methods. Ontario Ministry of the Environment, Water Research Branch, Rexdale, Ont.

Phillips, P.J., J.M. Denver, R.J. Shedlock, and P.A. Hamilton. 1993. Effects of forested wetlands on nitrate concentrations in ground water and surface water on the Delmarva Peninsula. Wetlands, 13: 75–83.

Renzetti, A.V.E., C.H. Taylor, and J.M. Buttle. 1992. Subsurface flow in a shallow soil Canadian Shield watershed. Nordic Hydrol. 23: 209–226.

Riley, J.L. 1988. Peat and peatland resources of southeastern Ontario. Mines and Minerals Division, Ontario Geological Survey, Ontario Ministry of Northern Development and Mines. Open File Rep. No. 5633.

Sparling, J.H. 1966. Studies on the relationship between water movement and water chemistry in mires. Can. J. Bot. 44: 747–758.

Urban, N.R., and S.E. Bayley. 1986. The acid–base balance of peatlands: a short-term perspective. Water Air Soil Pollut. 30: 791–800.

Urban, N.R, S.J. Eisenreich, and D.F. Grigal. 1989. Sulphur cycling in a forested sphagnum bog in northern Minnesota. Biogeochemistry, 7: 81–109.

Wieder, R.K. 1985. Peat and water chemistry at Big Run Bog, a peatland in the Appalachian mountains of West Virginia, USA. Biogeochemistry, 1: 277–302.

Winter, T.C. 1981. Uncertainties in estimating the water balance of lakes. Water Resour. Bull. 17: 82–115.

Oxygen consumption, nitrogenous excretion, and filtration rates of *Dreissena polymorpha* at acclimation temperatures between 20 and 32°C

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Abstract: Mussels were acclimated to each of four experimental temperatures (20, 24, 28, and 32°C) for 30 days. Mussels averaged 10.29 mg tissue dry weight. Oxygen consumption rates at 32°C were 3.65 times larger than consumption rates at 20°C ($p < 0.01$). Ammonia excretion rates at 32°C were 4.9 times greater than those at 20°C ($p < 0.01$). O:N ratios were >60 at 20 and 24°C but declined ($p < 0.01$) at 28 and 32°C to <40. Filtration rates, an estimate of feeding rates, were not significantly different at 20 and 24°C ($p > 0.50$) but declined at 28 and 32°C ($p < 0.01$). Filtration rates at 32°C were only 27% of the rates at 20°C. These results indicate that (*i*) the metabolic expenditure of *Dreissena polymorpha* rose 265% as the temperature rose from 20 to 32°C, (*ii*) metabolism relied more heavily on lipids and carbohydrates at 20 and 24°C while protein catabolism increased at 28 and 32°C, and (*iii*) the potential feeding rates of *D. polymorpha* declined by 73% as temperature rose from 20 to 32°C. Above 28°C *D. polymorpha* was unable to match energy expenditures with concurrent food intake and forced to rely on stored fuels.

Résumé : Nous avons acclimaté des moules à quatre températures d'expérimentation différentes (20, 24, 28 et 32°C) en les exposant à ces conditions pendant 30 jours. En moyenne, les moules comportaient 10,29 mg de tissus (à l'état sec). La consommation d'oxygène à 32°C était de 3,65 fois supérieure à celle mesurée à 20°C ($p < 0,01$). L'excrétion d'ammonium à 32°C était 4,9 fois supérieure à celle mesurée à 20°C ($p < 0,01$). Le rapport O/N était supérieur à 60 à 20 et 24°C, mais il a baissé ($p < 0,01$) à moins de 40 à 28 et 32°C. La vitesse de filtration, un indice de la vitesse d'alimentation, ne différait pas dans une mesure significative à 20 et 24°C ($p > 0,50$), mais elle a baissé à 28 et 32°C ($p < 0,01$). La vitesse de filtration à 32°C ne représentait que 27% de la valeur mesurée à 20°C. Ces résultats indiquent que (*i*) la dépense métabolique de *Dreissena polymorpha* a augmenté de 265% lorsque la température est passée de 20 à 32°C, (*ii*) le métabolisme dépendait plus des lipides et des glucides à 20 et 24°C et le catabolisme des protéines a augmenté à 28 et 32°C, et (*iii*) les valeurs possibles de la vitesse d'alimentation de *D. polymorpha* ont diminué de 73% lorsque la température est passée de 20 à 32°C. À plus de 28°C, *D. polymorpha* n'a pu combler ses dépenses énergétiques par l'alimentation et a dû puiser dans ses réserves.

[Traduit par la Rédaction]

Introduction

Since the first reports of its introduction in Lake St. Clair, Ontario (Hebert et al. 1989), in 1988 *Dreissena polymorpha* has successfully colonized all of the Great Lakes as well as many of the drainage systems of the central and northeastern United States (New York Sea Grant 1994). Because of its pelagic larvae and its ability to colonize and attach to hard substrates by byssal threads, *D. polymorpha* has become a major problem for users of untreated water (Mackie et al. 1989) as well as a major threat to native freshwater unionid mussels (Mackie 1991; Haag et al. 1993). Development of control strategies for water users (Griffiths et al. 1989) and determination of the ultimate geographic area in North America likely to be affected by *D. polymorpha* (Strayer 1991; Ramcharan et al. 1992; Schneider 1992) are major concerns. The temperature tolerance capabilities of *D. polymorpha* bear on both issues. Elevated temperature has been proposed as a control measure for *D. polymorpha* either alone or in combination with other control techniques (McMahon et al. 1994). Also, predictions of the ultimate distribution of *D. polymorpha* in southern North America have relied on presumed temperature tolerances (Strayer 1991). Unfortunately, very little information has been presented about the physiology of *D. polymorpha* above 20°C.

Received August 8, 1994. Accepted March 2, 1995.
J12523

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New information on the upper thermal tolerances of *D. polymorpha* would be useful for the development of control strategies and for predicting its likely geographic distribution in North America. This study reports the effects of temperature between 20 and 32°C on rates of oxygen consumption, nitrogenous excretion, and filtration in *D. polymorpha*.

Materials and methods

Mussels were obtained in early June from the U.S. Army Corp of Engineers Black Rock Lock Facility on Lake Erie, Buffalo, New York. After arrival in the laboratory, the mussels were placed in an LS-700 Living Stream at 20°C, fed dry *Chlorella* sp. (Nichols 1992), and kept under a 14 h light : 10 h dark cycle. Four acclimation groups of mussels with a shell length of 13–17 mm were set up on day 0 at 20, 24, 28, and 32°C. Acclimation temperatures above 20°C were established by increasing the temperature by 1°C/day until the correct temperature was reached. Mussels were fed dry *Chlorella* sp. daily except as noted below and maintained on the 14 h light : 10 h dark photoperiod cycle throughout the experiment.

On days 42 (mussels kept at 20 and 24°C) and 43 (mussels kept at 28 and 32°C), oxygen consumption rates were determined individually on 20 mussels from each of the acclimation groups at the acclimation temperature. Oxygen consumption was measured using YSI polarographic oxygen electrodes (YSI 5331), YSI meter 5300, YSI bath 5301, Lauda constant temperature circulator K-2/R, and a Fisher Recordall chart recorder. The YSI respirometer chambers were modified for use on small aquatic animals (Burky 1977). Prior to oxygen consumption determinations, mussels were not fed for 48 h to avoid the phenomenon of specific dynamic action (Bayne and Newell 1983). After oxygen consumption determinations, mussels were returned to feeding conditions for at least 24 h before starting the 24-h unfed period prior to ammonia excretion rate determinations.

On day 45 ammonia excretion rates were determined individually at the acclimation temperatures on the same mussels used to determine oxygen consumption rates. Ammonia excretion rates were determined by keeping individual mussels in known volumes of water at the appropriate temperature for 1.5 h and assaying the ammonia concentration using an Orion 9512 ammonia electrode coupled to an Orion 407RA ion meter. Mussels were not fed for 24 h prior to ammonia excretion determinations.

To integrate the data on oxygen consumption and ammonia excretion, O:N ratios were computed for each mussel used in this experiment by dividing its oxygen consumption rate in moles O by its ammonia excretion rate in moles N (Widdows 1978; Bayne and Newell 1983). O:N ratios are useful for assessing the relative contribution of protein to total catabolism (Bayne and Widdows 1978).

On day 46, filtration rates were determined individually for the same mussels at the acclimation temperature. Filtration rates were spectrophotometrically determined (Aldridge et al. 1987) for individual mussels in 1 L of water containing an initial dry *Chlorella* concentration of 80 mg/L. Each mussel was placed in 1 L of water to which the equivalent of 80 mg of dry *Chlorella* sp. was added volumetrically from a premixed suspension. Mussels were allowed to filter for 1.5 h. More *Chlorella* concentrate was added if, during this 1.5-h period, the concentration dropped below half of the starting value. After 1.5 h, each mussel was removed and the final *Chlorella* concentrations were determined by measuring optical densities at 720 nm using a Bausch & Lomb 710 spectrophotometer. Reductions in *Chlorella* concentrations relative to controls (*Chlorella* suspensions without mussels) were converted to filtration rates ($mg \cdot h^{-1}$) using an empirical relationship of optical density to *Chlorella* concentration.

For conceptual purposes, rates of oxygen consumption and filtration can be summarized in energetic terms ($J \cdot mg^{-1} \cdot h^{-1}$) using certain simplifying assumptions to give a useful energetic summary. The first two assumptions are that the O_2 consumption of our nonfeeding mussels was equivalent to the basal cost of maintenance and that 1 mL O_2 = 20.08 J (Bayne and Newell 1983). To estimate the energetic yield to the mussels of *Chlorella* sp. requires four assumptions: (*i*) mussel ingestion rates were 33% of filtration rates (Walz 1978*a*), (*ii*) mussel assimilation efficiency equaled 40% (Walz 1978a), (*iii*) the energetic cost of feeding equaled 24% of the ingested ration (Bayne and Newell 1983), and (*iv*) 1 mg dry *Chlorella* sp. equaled 21.08 J. Hence

$$\text{Ingestion rate (in } J \cdot mg^{-1} \cdot h^{-1}) = \text{filtration rate (in } mg \cdot mg^{-1} \cdot h^{-1}) \cdot 21.08\ J \cdot mg^{-1} \cdot 0.33$$

$$\text{Assimilation rate (in } J \cdot mg^{-1} \cdot h^{-1}) = \text{ingestion rate (in } J \cdot mg^{-1} \cdot h^{-1}) \cdot 0.40$$

$$\text{Energetic expenditure (in } J \cdot mg^{-1} \cdot h^{-1}) = (\text{oxygen consumption (in } mL \cdot mg^{-1} \cdot h^{-1}) \cdot 20.08\ J \cdot mL^{-1}) + (\text{ingestion rate (in } J \cdot mg^{-1} \cdot h^{-1}) \cdot 0.24)$$

$$\text{Scope for growth (in } J \cdot mg^{-1} \cdot h^{-1}) = \text{assimilation rate (in } J \cdot mg^{-1} \cdot h^{-1}) - \text{energetic expenditure (in } J \cdot mg^{-1} \cdot h^{-1})$$

At the end of the filtration rate determinations, mussels were fixed in 10% Formalin buffered with $CaCO_3$. On the next day the shells were decalcified in 10% HNO_3 and the soft tissues separated from the periostracum and byssal threads. The soft tissue was then rinsed with water to remove the acid, placed in preweighed boats, and dried to constant weight at 60°C to obtain tissue dry weights (TDW).

Results

Mean TDW for mussels from the 20, 24, 28, and 32°C acclimation groups were 10.89 ± 3.19 mg (mean ± SD, n = 19), 10.45 ± 2.77 mg (n = 19), 10.24 ± 3.26 mg (n = 20), and 9.59 ± 1.83 mg (n = 20), respectively. These means are not significantly different (one-way analysis of variance (ANOVA) with post hoc Tukey's honestly significant difference (HSD) test $p > 0.50$). Because of this and the limited range of mussel TDW values, all of the rate measures reported below are weight specific and were obtained by dividing the whole mussel rates by TDW.

Weight-specific rates of oxygen consumption, ammonia excretion, and filtration as well as the O:N ratios (arcsine transformed) were analyzed by ANOVA with acclimation temperature as the independent variable and TDW as the covariate. Acclimation temperature had a significant effect (ANOVA) on oxygen consumption rates (F ratio = 37.9,

Table 1. Rates of oxygen consumption, ammonia excretion, and filtration along with molar O:N ratios for *Dreissena polymorpha* after temperature acclimation at 20, 24, 28, and 32°C.

Temperature (°C)	Oxygen consumption ($\mu g\ O_2 \cdot mg^{-1} \cdot h^{-1}$)		Ammonia excretion ($\mu g\ NH_3 \cdot mg^{-1} \cdot h^{-1}$)		O:N ratio (moles O : moles N)		Filtration (mg *Chlorella*$\cdot mg^{-1} \cdot h^{-1}$)	
	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*
20	1.62±0.40	19	0.039±0.025	19	64±42	19	0.153±0.109	19
24	2.45±0.48	19	0.045±0.018	19	68±33	19	0.189±0.099	19
28	4.21±1.39	20	0.135±0.041	20	35±13	20	0.082±0.057	20
32	5.92±2.27	20	0.191±0.056	20	35±14	20	0.041±0.041	20

$p < 0.001$), ammonia excretion rates (F ratio = 71.4, $p < 0.001$), O:N ratio (F ratio = 8.6, $p < 0.001$), and filtration rates (F = 13.5, $p < 0.001$). TDW was a significant covariate for oxygen consumption rates (F ratio = 12.8, $p < 0.001$) and ammonia excretion rates (F ratio = 5.8, $p < 0.02$). Following the ANOVA analyses, pair-wise comparisons of the means at the acclimation temperatures were compared using Tukey's HSD multiple comparison test. The p values that follow report Tukey's HSD comparisons.

Mean oxygen consumption rates of mussels acclimated at 20, 24, 28, and 32°C are shown in Table 1 and Fig. 1*a*. Oxygen consumption rates for mussels at 20 and 24°C were not significantly different ($p > 0.25$, Tukey's HSD test). However, oxygen consumption rates rose significantly from 24 to 28°C and again from 28 to 32°C ($p < 0.001$). Oxygen consumption rates at 32°C were 3.65 times higher than rates for mussels at 20°C.

Mean ammonia excretion rates for mussels acclimated at 20, 24, 28, and 32°C are shown in Table 1 and Fig. 1*b*. Ammonia excretion rates for mussels acclimated to 20 and 24°C were not significantly different ($p > 0.97$, Tukey's HSD test). Ammonia excretion rates at 24 and 28°C were significantly different ($p < 0.001$) as were the rates at 28 and 32°C ($p < 0.001$). Between 20 and 32°C ammonia excretion increased by 390%.

Mean O:N ratios for mussels acclimated at 20, 24, 28, and 32°C are shown in Table 1 and Fig. 1*c*. O:N ratios at 20 and 24°C were not significantly different ($p > 0.97$, Tukey's HSD test) and the same is true between the O:N ratios at 28 and 32°C ($p = 1$). O:N ratios at 28 and 32°C, however, were significantly different from those at 20 and 24°C ($p < 0.001$). Using the computational methods of Aldridge et al. (1986) these ratios indicate that mussels acclimated to 20 and 24°C used protein to provide about 12% of their energy needs while mussels acclimated to 28 and 32°C used protein to meet 20% of their energy requirements.

Mean filtration rates of resuspended, dry *Chorella* sp. for mussels acclimated at 20, 24, 28, and 32°C are shown in Table 1 and Fig. 1*d*. Filtration rates at 20 and 24°C ($p > 0.56$, Tukey's HSD test) as well as at 28 and 32°C ($p > 0.35$) were not significantly different. Compared with filtration rates at 20°C, rates at 28°C ($p < 0.03$) and 32°C ($p < 0.001$) were significantly lower. Filtration rates at 24°C were also significantly higher than those at 28°C ($p < 0.001$) and 32°C ($p < 0.001$). Filtration declined by 78% as acclimation temperature rose from 24 to 32°C.

Figure 2 shows estimated weight-specific rates of metabolic expenditure, assimilation, and scope for growth at 20, 24, 28, and 32°C. At 28 and 32°C, *D. polymorpha* was unable to maintain a positive scope for growth.

Discussion

The weight-specific oxygen consumption rates of *D. polymorpha* reported here are comparable to values reported by other authors (Woynarovich 1961; Sprung 1991; Sprung and Borcherding 1991), although direct comparisons are complicated by differences in technique, size of animals, temperature, and acclimation regime, among other factors. For mussels acclimated above 15°C, McMahon and Alexander (1991) reported that O_2 consumption rates rise as temperature increases to 35°C, as was the case between 20 and 32°C in this study. There is no obvious evidence of temperature compensation of the basal oxygen consumption rate in nonfeeding *D. polymorpha* over this temperature range.

The weight-specific ammonia excretion rates reported in this study for mussels averaging a TDW of 10.29 mg are much lower than those reported by Sprung (1991) for sexually mature females with a TDW of 50 mg in spawning or prespawning condition. The pattern of low ammonia excretion in young molluscs compared with adults in reproductive condition was also observed in *Leptoxis carinata* (Bruguiére) (Aldridge 1985) where it was attributed to protein conservation by rapidly growing juveniles.

The O:N ratios of the mussels in the four acclimation groups indicate that at 20 and 24°C, the mussels were primarily catabolizing carbohydrates and lipids (O:N>50; Bayne and Widdows 1978). At 28 and 32°C, however, the mussels used relatively more protein in catabolism (O:N<40). Among molluscs such reductions in O:N ratios are usually associated with trophic stress (Bayne 1973; Widdows 1978; Bayne et al. 1979, 1981; Bayne and Newell 1983; Aldridge et al. 1987). In this study filtration rates peaked at 24°C and fell dramatically at 28 and 32°C. Other workers have reported maximal filtration rates between 15 and 20°C (Walz 1978*a*; Reeders and Bij De Vaate 1990), while Morton (1971) observed maximal filtration rates for *D. polymorpha* at 31°C at high particle concentrations.

The summary of the energy data presented in Fig. 2 suggests that over the 20–32°C temperature range, maximal rates of growth in *D. polymorpha* will be attained

Fig. 1. The means of (*a*) oxygen consumption, (*b*) ammonia excretion, (*c*) O:N ratios (by atomic equivalents), and (*d*) filtration rates for *Dreissena polymorpha* after temperature acclimation at 20, 24, 28, and 32°C. Bars delineate two standard errors and lines between means were fitted by distance-weighted least squares (Statistica 1994).

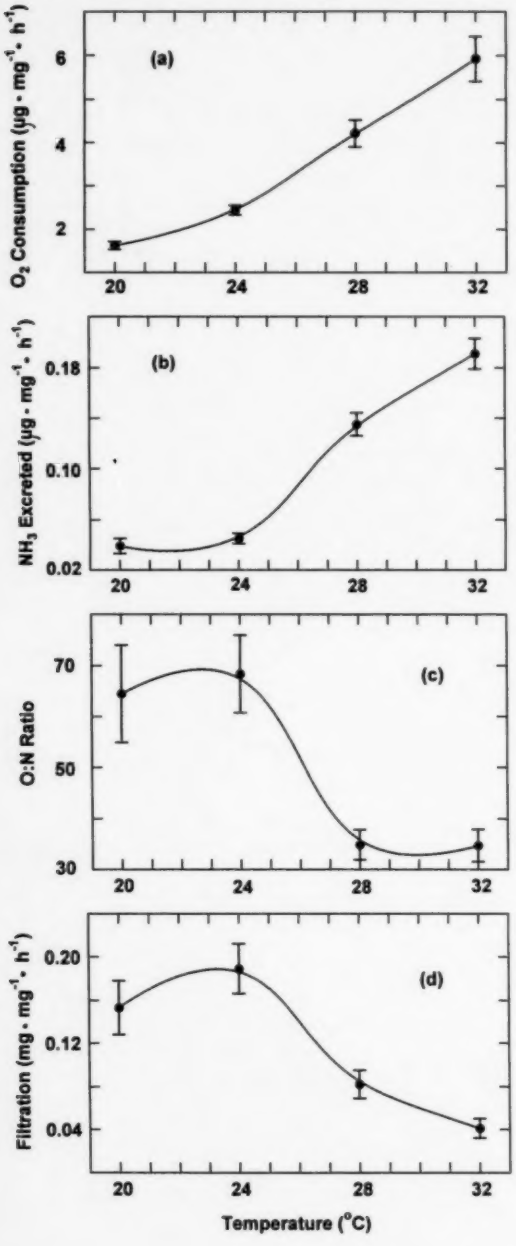


Fig. 2. Estimated average rates of assimilation (circles), energy expenditure (triangles), and scope for growth (squares) in the energy budgets of *Dreissena polymorpha* after temperature acclimation at 20, 24, 28, and 32°C. Lines between means were fitted by distance-weighted least squares (Statistica 1994).

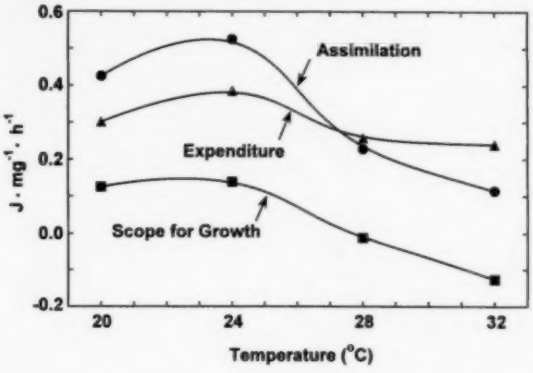
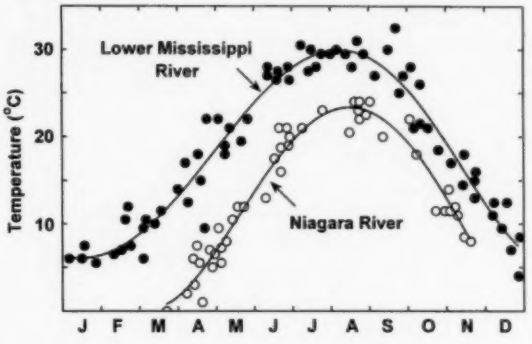


Fig. 3. Water temperatures for the lower Mississippi River in Louisiana (solid circles) and the Niagara River (open circles) from 1986 to 1993. Curves represent a fit to a fifth-order polynomial to each data set (Statistica 1994).



between 20 and 24°C, assuming adequate food availability. Walz (1978*b*) reported *D. polymorpha* growth rates to be maximal between 10 and 15°C for mussels acclimated to 8, 15, 20, and 25°C. The difference between his findings and ours is probably due to the sources of the mussels. Walz obtained his mussels from Lake Constance at 8 m depth where summer temperatures reach 20°C only rarely (Walz 1978*c*). Our mussels were collected from 2 m at Black Rock Lock on the Niagara River where summer temperatures generally exceed 20°C (Fig. 3).

The failure of *D. polymorpha* to maintain a positive scope for growth above 28°C is due to the inflation of its basal energetic expenditure beyond the capacity of its filter-feeding mechanism to compensate. Similar findings have been reported for *Donax vittatus* (da Costa) (Ansell and Sivadas 1973) and *Macoma balthica* (L.) (De Wilde 1975; Lammens 1967). Meeting the high basal metabolic expenditure appears to reduce the filtration capability of *D. polymorpha* to such an extent that at 28°C the estimated

assimilation rate is just below concurrent metabolic expenditure while at 32°C metabolic expenditure exceeds the estimated assimilation rate, thus forcing the mussels into negative growth (Bayne and Newell 1983).

Mussel size affects rates of oxygen consumption and filtration rates and, hence energetic losses and gains, in an allometric fashion (Bayne and Newell 1983) that makes extrapolating these results to other size-classes of *D. polymorpha* difficult. Given the higher weight-specific metabolic rates of smaller animals than larger ones, it might be expected that smaller *D. polymorpha* might be more adversely affected by higher temperatures than larger individuals, but McMahon et al. (1994) have shown that smaller *D. polymorpha* tolerate chronic exposure to high temperature better than larger individuals. Likewise, Bayne and Newell (1983) have shown that on the basis of the comparison of actual biomass changes with those predicted from measured rates of oxygen consumption in *Cerastoderma edule* (L.) and *Mytilus edulis* L., smaller individuals are better at reducing energetic losses during periods of food shortage than larger individuals. Hence, there is some evidence that suggests high temperatures may negatively affect the physiological energetics of large mussels more than smaller ones.

In the present study, zebra mussels collected from the Niagara River were exceptionally tolerant of moderately high temperatures. Mussels acclimated at 32°C survived for 35 days without extensive mortality. McMahon et al. (1995) estimated a mean tolerance time of 150 h at 32°C for *D. polymorpha* collected from the Niagara River and acclimated at 30°C. Iwanyzki and McCauley (1993), working with *D. polymorpha* from lakes Erie and St. Clair and acclimated at 25°C, measured a mean tolerance time of just 95 h at 30°C. In general, North American *D. polymorpha* appear to have originated from a thermally tolerant race from the southern portion of this species' European range (McMahon et al. 1995).

The degree of thermal tolerance observed in the present study is concordant with the present distribution of *D. polymorpha* in North America, which includes populations throughout the lower Mississippi River as far south as Louisiana (New York Sea Grant 1994). According to annual reports of the United States Geological Survey (1986–1993*a*), temperatures of the lower Mississippi River in Louisiana tend to reach a maximum of 30°C in summer (Fig. 3). The maximum temperature recorded during these years was 32.5°C. Our results indicate that at temperatures above 28°C energy assimilation rates cannot keep pace with energetic expenditure. Temperatures exceed 28°C for approximately 75 days during summer in the lower Mississippi River in Louisiana. In contrast, the temperature of the Niagara River (the source of mussels used in our study and a location with a temperature profile generally representative of lakes Erie and Ontario in which *D. polymorpha* thrives) averages 23°C in summer (U.S. Geological Survey 1986–1993*b*) (Fig. 3). The maximum temperature recorded during these years was 24.5°C.

In addition to a negative scope for growth at high temperature, growth of *D. polymorpha* ceases when the temperature drops below a minimum value of approximately 9°C. Estimates of the minimum temperature required for growth range from 6 to 12°C, with the threshold for growth of Lake St. Clair populations ranging from 8 to 10°C (Claudi and Mackie 1994 and references therein). For approximately 80 days in winter, the temperature of the lower Mississippi River in Louisiana generally ranges from 6 to 9°C (U.S. Geological Survey 1986–1993*a*) (Fig. 3) The lowest temperature recorded in any of these years was 4°C. In contrast, the temperature of the Niagara River tends to range from 0 to 9°C for approximately 175 days from late fall to mid spring, typically including a 120-day period with temperatures less than 4°C (U.S. Geological Survey 1986–1993*b*) (Fig. 3).

Hence, the summer period of negative scope for growth endured by southern populations of *D. polymorpha* is counteracted by a much shorter and less severe period of winter growth stress than is experienced by northern populations. Assuming that growth is largely restricted to a temperature range of 9–28°C and ignoring confounding effects of reproductive activity and food availability, maximum growth of individuals in southern populations will occur from late winter to early summer and again from late summer to late fall, with periods of growth stress likely to be encountered in midsummer and winter. This contrasts with an expected pattern of maximum growth from midspring to midfall and of no growth or degrowth from late fall to early spring in the Great Lakes region.

In summary, the data reported in this study indicate that medium-sized *D. polymorpha* become unable to meet their immediate metabolic expenditure with concurrent intake above 28°C. The O:N ratios and filtration rates as well as the scope for growth indicate that mussels at 28 and 32°C experience moderate trophic stress. Regarding the ultimate geographic distribution for *D. polymorpha* in North America these data are congruent with earlier predictions (Strayer 1991) and the current reality (New York Sea Grant 1994). Both our results and recent reports of *D. polymorpha* at sites scattered throughout the lower Mississippi River (New York Sea Grant 1994) suggest that this species ultimately will occur over most of the southern United States. However, it remains to be seen if the populations of *D. polymorpha* in the southern United States will achieve the ecological success of populations in the Great Lakes region or whether the bioenergetic stress of moderately high but sublethal summer temperatures will prevent the southernmost populations from thriving.

Acknowledgments

This study was conducted as part of the Zebra Mussel Research Program of the U.S. Army Corp of Engineers (contract DACW39-92-K-0022). Permission to publish this paper was granted by the Chief of Engineers. We gratefully thank Gary L. Dye of the U.S. Army Corp of Engineers for supplying mussels. We also thank Sheeré J. Mills for assistance with the manuscript.

References

Aldridge, D.W. 1985. Seasonal patterns of carbon and nitrogen partitioning in the freshwater prosobranch *Leptoxis carinata*. Am. Malacol. Bull. 3: 169–177.

Aldridge, D.W., W.D. Russell-Hunter, and D.E. Buckley. 1986. Age-related differential catabolism in the snail, *Viviparus georgianus*, and its significance in the bioenergetics of sexual dimorphism. Can. J. Zool. 64: 340–346.

Aldridge, D.W., B.S. Payne, and A.C. Miller. 1987. The effects of intermittent exposure to suspended solids and turbulence on three species of freshwater mussels. Environ. Pollut. 45: 17–28.

Ansell, A.D., and P. Sivadas. 1973. Some effects of temperature and starvation on the bivalve *Donax vittatus* (da Costa) in experimental laboratory populations. J. Exp. Mar. Biol. Ecol. 13: 229–262.

Bayne, B.L. 1973. Aspects of the metabolism of *Mytilus edulis* during starvation. Neth. J. Sea Res. 7: 399–410.

Bayne, B.L., and R.C. Newell. 1983. Physiological energetics of marine molluscs. *In* The Mollusca. Vol. 4. Physiology. Part 1. *Edited by* K.M. Wilbur and A.S.M. Saleuddin. Academic Press, New York. pp. 407–515.

Bayne, B.L., and J. Widdows. 1978. The physiological ecology of two populations of *Mytilus edulis* L. Oecologia, 37: 137–162.

Bayne, B.L., M.N. Moore, J. Widdows, D.R. Livingstone, and P. Salkeld. 1979. Measurements of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs. Philos. Trans. R. Soc. London Ser. B, 286: 563–581.

Bayne, B.L., K.R. Clarke, and M.N. Moore. 1981. Some practical considerations in the measurement of pollution effects on bivalve molluscs, and some possible ecological consequences. Aquat. Toxicol. 1: 159–174.

Burky, A.J. 1977. Respiration chambers for measuring oxygen consumption of small aquatic molluscs with Clark-type polarographic electrodes. Malacol. Rev. 10: 71–72.

Claudi, R., and G.L. Mackie. 1994. Practical manual for zebra mussel monitoring and control. Lewis Publishers, Boca Raton, Fla.

De Wilde, P.A.W.J. 1975. Influence of temperature on behavior, energy metabolism and growth of *Macoma balthica* (L.). *In* Proceedings of the 9th European Marine Biology Symposium, Dunstaffinage Marine Research Laboratory, Oban, Scotland, Oct. 2–8, 1974. *Edited by* H. Barnes. Aberdeen University Press, Aberdeen, Scotland. pp. 239–256.

Griffiths, R.W., W.P. Kovalak, and D.W. Schloesser. 1989. The zebra mussel, *Dreissena polymorpha* (Pallas, 1771), in North America: impact on raw water users. *In* Symposium: service water system problems affecting safety-related equipment. Electric Power Research Institute, Nuclear Power Division, Palo Alto, Calif.

Haag, W.R., D.J. Berg, D.W. Garton, and J.L. Farris. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. Can. J. Fish. Aquat. Sci. 50: 13–19.

Hebert, P.D.N., B.W. Muncaster, and G.L. Mackie. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes region. Can. J. Fish. Aquat. Sci. 46: 1587–1591.

Iwanyzki, S., and R.W. McCauley. 1993. Upper lethal temperatures of adult zebra mussels (*Dreissena polymorpha*). *In* Zebra mussels: biology, impacts, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 667–673.

Lammens, J.J. 1967. Growth and reproduction of a tidal flat population of *Macoma balthica* (L.). Neth. J. Sea Res. 3: 315–382.

Mackie, G.L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha*, in relation to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia, 219: 251–268.

Mackie, G.L., W.N. Gibbons, B.W. Muncaster, and I.M. Gray 1989. The zebra mussel, *Dreissena polymorpha*: a synthesis of European experiences and a preview for North America. Report prepared for Water Resources Branch, Great Lakes Section, Department of Energy, Mines, and Resources. Available from Queen's Printer for Ontario, ISBN 0-7729-5647-2.

McMahon, R.F., and J.E. Alexander, Jr. 1991. Respiratory responses to temperature, hypoxia and temperature acclimation in the zebra mussel, *Dreissena polymorpha* (Pallas). Am. Zool. 31: 74A.

McMahon, R.F., M.A. Matthews, T.A. Ussery, R. Chase, M. Clarke, and T.H. Dietz. 1994. Further studies of heat tolerance in zebra mussels: effects of chronic lethal temperatures and geographic distribution on survival times. *In* Proceedings of the 4th International Zebra Mussel Conference, Madison, Wis., March 7–10, 1994. University of Wisconsin Sea Grant, Madison, Wis. pp. 251–272.

McMahon, R.F., M.A. Matthews, T.A. Ussery, R. Chase, and M. Clarke. 1995. Studies of heat tolerance in zebra mussels: effects of temperature acclimation and chronic exposure to lethal temperatures. U.S. Army Corp of Engineers Waterways Experiment Station Technical Report No. EL-95-9.

Morton, B.S. 1971. Studies on the biology of *Dreissena polymorpha* Pall. V. Some aspects of filter-feeding and the effect of microorganisms upon the rate of filtration. Proc. Malacol. Soc. Lond. 39: 289–301.

New York Sea Grant. 1994. North American range of the zebra mussel. *In Dreissena polymorpha* information review No. 5. Zebra Mussel Information Clearing House, New York Sea Grant, Brockport, N.Y. pp. 8–9.

Nichols, S.J. 1992. Maintenance of the zebra mussel (*Dreissena polymorpha*) under laboratory conditions. *In* Zebra mussels: biology, impacts, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton, Fla.

Ramcharan, C.W., D.K. Padilla, and S.I. Dodson. 1992. Models to predict potential occurrence and density of the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 49: 2611–2620.

Reeders, H.H., and A. Bij De Vaate. 1990. Zebra mussels (*Dreissena polymorpha*): a new perspective for water quality management. Hydrobiologia, 200–201: 437–450.

Schneider, D.W. 1992. A bioenergetics model of zebra mussel, *Dreissena polymorpha*, growth in the Great Lakes. Can. J. Fish. Aquat. Sci. 49: 1406–1416.

Sprung, M. 1991. Expenditure of reproduction: a study on metabolic requirements of the gonads and fecundity of the bivalve *Dreissena polymorpha*. Malacologia, 33: 63–70.

Sprung, M., and J. Borcherding. 1991. Physiological and morphometric changes in *Dreissena polymorpha* (Mollusca; Bivalvia) during a starvation period. Malacologia, 33: 179–191.

Statistica. 1994. Distance-weighted least squares fitting. *In* Statistica for Windows. Vol. 2. Graphics. Statsoft Inc., Tulsa, Okla. p. GRA-2677.

Strayer, D.L. 1991. Projected distribution of the zebra mussel, *Dreissena polymorpha*, in North America. Can. J. Fish Aquat. Sci. 48: 1389–1395.

U.S. Geological Survey. 1986–1993*a*. Water sources data, Louisiana, water years 1986–1993. *In* U.S. Geological Survey water data reports LA-86-1 to LA 93-1. U.S. Geological Survey, Baton Rouge, La.

U.S. Geological Survey. 1986–1993*b*. Water resources data, western New York, water years 1986–1993. U.S. Geological Survey water data reports NY-86-3 to NY-93-3. U.S. Geological Survey, Albany, N.Y.

Walz, N. 1978*a*. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in the laboratory experiments and in Lake Constance. I. Pattern of activity, feeding and assimilation efficiency. Arch. Hydrobiol. Suppl. 55: 83–105.

Walz, N. 1978*b*. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. III. Growth under standard conditions. Arch. Hydrobiol. Suppl. 55: 121–141.

Walz, N. 1978*c*. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. IV. Growth in Lake Constance. Arch. Hydrobiol. Suppl. 55: 142–156.

Widdows, J. 1978. Physiological indices of stress in *Mytilus edulis*. J. Mar. Biol. Assoc. U.K. 58: 125–142.

Woynarovich, E. 1961. The oxygen consumption of *Dreissena polymorpha* (Lamellibranchiata) at different temperatures. Ann. Biol. Tihany, 28: 211–216.

A model for the determination of fish species at risk based upon life-history traits and ecological data

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Abstract: Fish species at risk (n = 29) and not at risk (n = 88) of extinction in the Great Lakes – St. Lawrence biozone were compared to determine if they could be distinguished by their life-history characteristics. A matrix of 51 variables representing 2 phylogenetic, 18 life-history, and 31 ecological variables was compiled from literature sources. Nonparametric analysis shows that 14 variables distinguish between species at risk and not at risk. Logistic regression indicates that seven factors are significant in defining the two groups: age at maturation, feeding habitat, fish feeding regime, feeding substrate, water flow over feeding area, breeding habitat, and breeding substrate. The resulting model is concordant at 97.1% (p = 0.0001, −2 log likelihood = 38.9). The model states that any fish species maturing at 18 years or more is at risk of extinction in the biozone. When applied to a set of fish species from the American Midwest using a decision level of p = 49%, the model had a reclassification success of 77.8%. The modeling technique could be useful to evaluate species at risk and to complement decision making with regard to vulnerability, especially in the case of wide-ranging species such as Atlantic sturgeon (*Acipenser oxyrhynchus*) and American eel (*Anguilla rostrata*).

Résumé : Nous avons comparé 51 caractéristiques biologiques des espèces de poissons menacés (n = 29) ou non (n = 88) d'extinction dans la biozone des Grands-Lacs et du fleuve Saint-Laurent pour voir ce qui distinguait ces deux groupes. La matrice, compilée à partir de divers ouvrages, comprend 2 variables phylogénétiques, 18 variables du cycle vital et 31 variables écologiques. L'analyse non-paramétrique montre que 14 variables distinguent les deux groupes. La régression logistique indique que sept facteurs sont significatifs pour définir les deux groupes : l'âge à la maturité, le degré d'ichtyophagie, l'habitat et le substrat d'alimentation, la vitesse du courant dans l'aire d'alimentation, l'habitat et le substrat de reproduction. La concordance du modèle résultant est de 97,1% (p = 0,0001, −2 log likelihood = 38,9). Le modèle indique que toutes les espèces matures à 18 ans ou plus risquent l'extinction dans la biozone. Lorsqu'appliqué avec un seuil décision p = 49% à un jeu de données du Midwest américain, le modèle a eu un succès de reclassification de 77,8%. Cette technique de modélisation pourrait aider à évaluer le risque d'extinction ou la vulnérabilité de certaines espèces, notamment des poissons à distribution étendue tels l'Esturgeon noir (*Acipenser oxyrhynchus*) et l'Anguille d'Amérique (*Anguilla rostrata*).

Introduction

With accelerating disturbance of natural ecosystems by habitat alteration and introduction of exotic species, predictive models are needed that can be used in preservation and conservation programs (Lande 1988). Partridge and Harvey (1988) found that life histories are good predictors of populations' responses to changed environments. They showed that life histories evolve largely in response to the impact of different environments on the survival and fertility of different age-classes. As we shall see, modeling life-history traits and ecological data may also be useful in predicting the ways in which a fish community responds to environmental stresses.

In the Great Lakes – St. Lawrence biozone (Fig. 1), many fish species are currently at risk of becoming extinct as the result of human activity. We refer to these vulnerable species as being at risk of extinction in the biozone. Until 200 years ago, the fish community of the Great Lakes – St. Lawrence biozone was primarily composed of large, long-lived, K-selected species, such as lake sturgeon (*Acipenser fulvescens*) and lake trout (*Salvelinus namaycush*) (Regier 1979). However, more recently higher levels of ecosystem disturbance (e.g., overfishing, eutrophication, habitat destruction, fish invasions) have resulted in a large decline in abundance and diversity of native fish communities (Francis et al. 1979). Human activities that disrupt the structure of fish communities in turn result in these same communities becoming vulnerable to invasions by new fish species. Ten fish species have invaded the Canadian Great Lakes basin since 1949 (Mandrak 1989).

Received August 12, 1994. Accepted March 3, 1995.
J12535

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Fig. 1. Map of the Great Lakes – St. Lawrence and surrounding biozones (partly after Burnett et al. 1989). 1, Marine Coastal; 2, Boreal; 3, Prairie; 4, Great Lakes – St. Lawrence; 5, Atlantic Maritime; 6, Transition; 7, Carolinean.

Invading species take advantage of these disturbances by taking over the niches of recently extinct species or niches of the members of the existing community. The native fish communities are then negatively affected as they face increased competition for breeding space and food resources. As a result of these ecosystem and community disturbances, native fish communities in the Great Lakes – St. Lawrence biozone are increasingly at risk of extinction. In the last two centuries, the native fish communities of the Great Lakes – St. Lawrence biozone have been transformed into communities characterized by decreased diversity of small, short-lived, r-selected pelagic species (Paloheimo and Regier 1982).

In the last 10 years, U.S. and Canadian governmental agencies have been concerned with determining which species in North America are at risk of extinction, i.e., vulnerable, threatened, endangered, or extirpated (for definitions, see either Campbell 1988 or Burnett et al. 1989). These determinations have been based mainly on two factors (Burnett et al. 1989): (*i*) reduction in abundance and (*ii*) decreased geographic range, or restricted range. Lists of species at risk have been prepared by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (Campbell 1993), the American Fisheries Society (AFS) (Williams et al. 1989), and the Quebec Fish and Game Department (Beaulieu 1993). COSEWIC is comprised of wildlife conservation agencies of the federal, provincial, and territorial governments in Canada as well as three nongovernmental organizations: the World Wildlife Fund Canada, the Canadian Nature Federation, and the Canadian Wildlife Federation. In this study,

Table 1. Description of the 51 variables used in the analysis (codes are given in parentheses).

	Wilcoxon score
Phylogenetic variables	
(Order)	No test
(Family)	No test
Life-history traits	
Average length (in cm) reached by the female (Length)	0.8054
Age in years at which 50% or more of the females are mature (Agemat)	0.1753
Maximum normal age of the females in years (Lifespan)	0.6320
Migratrory behavior: 0 for nonmigratory or catadromous species, 1 for anadromous or potamodromous species (Migrant)	0.1459
Feeding behavior: 1 when species feeds in schools or in groups with no territory mentioned, otherwise 0 (FSchool)	0.7969
Breeding behavior: 1 when species spawns in groups with no territory mentioned, otherwise 0 (BSchool)	0.4247
Season (0 = winter, 1 = spring, 2 = summer, 3 = fall) at which spawning begins (Season)	0.1398
Spawning season in days: precise number if available, otherwise 15 d for 0–30 d , 45 d for 1–2 months and 75 d if more than 2 months (Duration)	0.7160
Food: each food item must comprise a minimum of 25% of adult feeding	
Fish (Piscivor)	0.0380
Algae or grass (Herbivor)	0.2808
Detritus (Detrivor)	0.9904
Invertebrates (Invert)	0.6018
Nesting behavior: 0 if none, 1 if yes (Nest)	0.0281
Parental care after birth only: 0 if none, 1 if yes (Parcare)	0.0144
Mean range of clutch sizes reported for a population (Clutch)	0.5614
Mean diameter (to nearest 0.1 mm) of fully yolked ovarian oocytes (Eggsize)	0.1225
Number of breeding seasons: usually life-span − age at maturity + 1 (Breeds)	0.6521
Predators: 0 if they are fish, 1 if they are bear, eagle, toothed whale or man (Predator)	0.9796
Ecological variables	
Presence or absence in the adjoining Boreal biozone (Boreal)	0.0001
Feeding habitat	
Lakes (FLake)	0.0007
Ponds (FPond)	0.0178
Rivers (FRiver)	0.0429
Streams (FStream)	0.0984
Wetlands and marshes (FWetmar)	0.8589
Deep waters (over 30 m, FDepth)	0.1871
Estuaries and seas (FSea)	0.8589
Feeding substrate	
Bedrock (FRock)	0.5581
Gravel or pebbles (FGrapeb)	0.2583
Sand (FSand)	0.5555
Mud or silt (FMudsil)	0.8583
Grasses (FGrass)	0.0003
Feeding level: 0 on bottom, 1 in midwater (FLevel)	0.2700
Speed of current over feeding area: 1 for moderate to fast water flow, 0 for slow or still water (FFlow)	0.0328
Breeding habitat	
Lakes (BLake)	0.0417
Ponds (BPond)	0.0137
Rivers (BRiver)	0.1663
Streams (BStream)	0.0475
Wetlands and marshes (BWetmar)	0.6510
Deep waters (over 30 m, BDepth)	0.0018
Estuaries and seas (BSea)	0.5794

Table 1 (*concluded*).

	Wilcoxon score
Breeding substrate	
Bedrock (BRock)	0.6013
Gravel or pebbles (BGrapeb)	0.0100
Sand (BSand)	0.8322
Mud or silt (BMudsil)	0.1122
Grasses (BGrass)	0.2426
Lowest temperature at which spawning starts (usually) or ends (Spawtemp)	0.3882
Breeding level: 0 on bottom, 1 in midwater (Blevel)	0.3151
Speed of current over breeding area: 1 for moderate to fast water flow, 0 for slow or still water (BFlow)	0.1059
Water turbidity: 0 if found only in clear water, 1 if also found in turbid waters (Turbid)	0.1447

Note: The right column gives the Wilcoxon score of the nonparametric univariate analysis for each variable, when it was compared with the status of 117 species of fish at risk or not at risk (Table 2). Variables with scores less than 0.05 are significantly different between species at risk and those not at risk.

no life-history traits or ecological data that either correlated with or were direct measures of abundance or geographic distribution of the fish species were used for the analysis. In this way, we made sure that none of the variables tested would overlap with those used by COSEWIC and AFS in their determination of which species are at risk of extinction. Current lists from these agencies include 20 vulnerable, 8 threatened, and 4 extinct or extirpated fish species for the Great Lakes – St. Lawrence biozone. This represents 25% of the total number of fish species living in this biozone (Campbell 1993).

Only in the last 5 years, with more research being focused on the status of endangered species, has the determination of a species' status actually reflected the current status of the species rather than its status 2 or 3 years previous to publication of the lists. The greater accuracy of these lists today increases the likelihood that the at risk lists will reflect those species affected by human activity.

In this paper, we analysed the fish fauna of the Great Lakes – St. Lawrence biozone. We included only the native species of the Great Lakes – St. Lawrence biozone and excluded those species that were invaders, were introduced, or whose status was undetermined at this time. The Great Lakes – St. Lawrence biozone includes many ecological zones such as the Great Lakes, the St. Lawrence River, and their tributaries. However, it also represents a single Atlantic drainage basin with a large faunal homogeneity. The native fish species in this biozone vary greatly in their life-history and ecological traits and have been subjected to intense ecological disturbances across the entire biozone for almost a century. As a result, this region is ideal for examining the possibility of a strong link between life-history traits and ecological data, and risk of extinction as a result of anthropocentric stresses.

We hypothesize that at risk and not at risk species of the Great Lakes – St. Lawrence biozone exhibit two distinct sets of life-history and ecological traits. Since current life-history theory (Stearns 1976) states that r-selected traits should be favored in unstable environments, we predict that at risk species should have traits found in K-selected species, i.e., large size, long life, long generation time, low fecundity, and slow growth. From a hydrographic point of view, the Great Lakes – St. Lawrence biozone may look like a stable environment, but from an ecological point of view, with the continuous modification of habitat and community structure, it is in fact an unstable environment for the native fish species. We also predict that migratory species, either anadromous or strongly potamodromous, are more affected than nonmigratory species because of damming, farming, and urbanization. We would not expect catadromous species to be affected in the same way, since they are able to change their choice of feeding sites, streams or rivers, when one becomes unusable through damming or unsuitable through chemical pollution. Moreover, since invaders are warmwater species (Mandrak 1989), we predict that species at risk will be mostly adapted to cold water. Finally, Mandrak (1989) suggested that invading success is also associated with omnivory and an intermediate food web level. Following Mandrak's (1989) suggestion, we predict that at risk species should be generalist feeders of intermediate food web level.

Through statistical analysis we defined the profile of life-history traits for both at risk and not at risk species and looked at the differences between the two groups. Several models were compared for this data set using their concordance and the log likelihood criterion, to find the model that best defined the differences between the two groups. This model was then applied to a set of fish species from the American Midwest to assess its applicability.

Materials and methods

Phylogeny and ecological data set[1]

Data were obtained for 51 variables: 2 phylogenetic, 18 life-history, and 31 ecological variables (Table 1). All qualitative variables were treated in a binomial way. We followed Lee et al.'s (1980) phylogeny of North American fishes.

[1] A database listing our principal literature sources, ecological traits, and numerical estimates is available, for a nominal fee, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada.

Families and orders were coded as categorical variables. Values of fish life-history traits and ecological data were obtained from literature sources that summarize large amounts of quantitative data for individual species (e.g., Scott and Crossman 1973; Lee et al. 1980; Becker 1983).

We used the life-history traits most commonly mentioned in textbooks on life-history theories (e.g., Begon et al. 1986). Since most fish species exhibit considerable variation in life-history traits over their geographical ranges, we used the average or modal values of traits from populations located in the biozone or as near to it as possible. In some cases, when no reliable data were found for a given trait, either no value was given or, in a few cases, we estimated the value by comparison with a second trait, which was related to the trait of interest, in a closely related species (e.g., egg size of species 1 was said to be the same as egg size of species 2 because both have the same prolarval size which is linked to egg size). Whenever maturation and growth data were reported for the sexes separately, we used the estimates for females to avoid differences being solely due to sex (Winemiller and Rose 1992). For species, such as the marine lamprey (*Petromyzon marinus*), having both migratory and land-locked populations, we used the data of the latter.

We used different ecological variables to describe the feeding and the breeding habitats of each fish species (Table 1). Presence of the species in the adjoining cool Boreal biozone was used as an ecological datum to test the hypothesis that species at risk are mostly adapted to cold water and thus most likely to be found also in that biozone. Since fish species often feed and breed in more than one habitat or over more than one substrate, we used as many binary variables as there were levels. Variables within a habitat or substrate group were therefore not mutually exclusive. For example, no feeding substrate was assigned to nonparasitic lampreys.

Selection of fish species

All species and subspecies found in the Great Lakes – St. Lawrence biozone by Scott and Crossman (1973), Lee et al. (1980), and McAllister et al. (1985) were considered for this analysis, including extinct species. We have included four extirpated species (Miller et al. 1989), because only a time factor separates them from the endangered status. These four species are the gravel chub (*Erimystax x-punctata*), the paddlefish (*Polyodon spathula*), the deepwater cisco (*Coregonus johannae*), and the longjaw cisco (*Coregonus alpenae*) (Table 2). Their inclusion in the analysis should help in defining traits that distinguish at risk species. We considered 150 species and subspecies, including anadromous and catadromous species, as well as some typical estuarine species such as the fourspine stickleback (*Apeltes quadracus*).

From our analysis, we removed all nonendemic and nonnative fishes of the Great Lakes – St. Lawrence biozone. Although COSEWIC lists the invading species as at risk because of their reduced geographic range in the biozone, we excluded them from our analysis because their life-history traits do not necessarily reflect traits that would cause a species to be at risk in the Great Lakes – St. Lawrence biozone. We removed from the species list all three European introduced species, the common carp (*Cyprinus carpio*), the brown trout (*Salmo trutta*), and the goldfish (*Carassius auratus*), and the six species of Pacific salmons and trouts that have been transplanted into the Great Lakes – St. Lawrence biozone by humans. Moreover, the invading redfin pickerel (*Esox americanus americanus*), having been found in the biozone only since 1944, was removed from the analysis.

We also removed from the list 10 species that are occasional visitors to Canadian waters along with species suspected to have been either recently introduced, or recently invading (Mandrak 1989) (Table 3). However, since the white perch (*Morone chrysops*) is an invader in the St. Lawrence River but is native to the Great Lakes, it was included in the analysis.

We did not consider subspecies, such as the blue walleye (*Stizostedion vitreum glaucum*) or grass pickerel (*Esox americanus vermiculatus*), because they share the same general life-history traits as their sibling subspecies, the yellow walleye (*Stizostedion vitreum vitreum*) and the redfin pickerel (*Esox americanus americanus*). Also, we did not consider the spring cisco (*Coregonus* sp.) and the pygmy smelt (*Osmerus spectrum*) as valid species. Both have only been recently considered as species, and they share the same general life-history traits as their mother species (*Coregonus artedi* and *Osmerus mordax*). Otherwise, we used the species definition as in Campbell (1993).

Status of fish species

The final list of fish species was divided into two groups according to their status. We followed the status assigned to fishes by COSEWIC (Campbell 1993) with four notable exceptions. In our list of species at risk we included striped bass (*Morone saxatilis*), lake sturgeon (*Acipenser fulvescens*), Atlantic sturgeon (*Acipenser oxyrhynchus*), and eastern sand darter (*Ammocrypta pellucida*). Because striped bass is highly endangered, if not extirpated, from the St. Lawrence (Beaulieu 1993), and since the COSEWIC status for it is still pending, we gave the species a threatened status (Table 2). Both Atlantic and lake sturgeons are listed as potentially at risk by the Quebec Fish and Game Department and have a status pending at COSEWIC. The AFS considers lake sturgeon as being threatened in Canada and Altantic sturgeon to be of special concern (equivalent to vulnerable). Both species are under severe stress in Quebec (R. Fortin, personal communication, Département de biologie, Université du Québec à Montréal, C.P. 8888, succursale A, Montréal, QC H3C 3P8, Canada). We therefore treated both species as vulnerable species. Finally, the eastern sand darter has not been seen for 25 years in Ontario (Scott and Crossman 1973), is very rare in Quebec, and is threatened in all adjacent American states (Williams et al. 1989). The species should soon receive vulnerable status at least (Campbell 1993). Therefore, we treated it as vulnerable in our analysis.

Ten fish species have a status pending at COSEWIC or are listed as potentially at risk by the Quebec Fish and Game Department (Table 2). We do not know if they are currently at risk, affected by cultural stresses, or not. They were removed from the list of species considered in the present analysis.

Table 2. Probability of being at risk (p %) of fishes native to the Great Lakes – St. Lawrence biozone (according to model 2).

Common name	Scientific name	p %	Status[a]	FGrass	BGrapeb	FLake	BStream	Piscivor	Agemat	FFlow
Species used to build the model										
Northern brook lamprey	*Ichthtomyzon fossor*	33.0	2	0	1	0	1	0	7	0
Silver lamprey	*Ichthyomyzon unicuspis*	0.0	1	1	1	1	0	1	6	0
American brook lamprey	*Lampetra lamottei*	12.8	1	0	1	0	1	0	5	0
Sea lamprey	*Petromyzon marinus*	0.0	1	0	1	1	1	1	7	0
Lake sturgeon	*Acipenser fulvescens*	97.3	2	0	1	1	0	1	23	0
Atlantic sturgeon	*Acipenser oxyrhynchus*	100.0	2	0	1	0	0	1	27	0
Paddlefish	*Polyodon spathula*	65.8	5	0	1	1	0	0	9	0
Spotted gar	*Lepisosteus oculatus*	0.4	2	1	0	1	0	1	4	0
Longnose gar	*Lepisosteus osseus*	0.4	1	1	0	1	0	1	4	0
Bowfin	*Amia calva*	0.4	1	1	0	1	0	1	4	0
Alewife	*Alosa pseudohanrengus*	4.8	1	0	1	1	0	0	3	0
American shad	*Alosa sapidissima*	12.8	1	0	1	0	1	0	5	0
Gizzard shad	*Dorosoma cepedianum*	0.1	1	1	1	1	0	0	2	0
Mooneye	*Hiodon tergisus*	0.0	1	0	1	1	1	1	5	1
Atlantic salmon	*Salmo salar*	27.5	1	0	1	0	0	1	4.5	0
Brook trout	*Salvelinus fontinalis*	0.0	1	0	1	0	1	1	3	0
Lake trout	*Salvelinus namaycush*	0.2	1	0	1	1	0	1	6.5	0
Longjaw cisco	*Coregonus alpenae*	95.1	5	0	0	1	0	0	3.5	0
Bloater	*Coregonus hoyi*	4.8	1	0	1	1	0	0	3	0
Deepwater cisco	*Coregonus johannae*	93.5	5	0	0	1	0	0	3	0
Kiyi	*Coregonus kiyi*	93.5	2	0	0	1	0	0	3	0
Blackfin cisco	*Coregonus nigripinnis*	96.3	3	0	0	1	0	0	4	0
Shortnose cisco	*Coregonus reighardi*	93.5	3	0	0	1	0	0	3	0
Shortjaw cisco	*Coregonus zenithicus*	98.0	3	0	0	1	0	0	5	0
Pigmy shitefish	*Prosopium coulteri*	0.0	1	0	1	1	1	0	4	0
Round whitefish	*Prosopium cylindraceum*	4.8	1	0	1	1	0	0	3	0
Rainbow smelt	*Osmerus mordax*	0.0	1	0	1	1	1	0	3	0
Central mudminnow	*Umbra limi*	19.3	1	1	0	0	1	0	2	0
Northern pike	*Esox lucius*	0.3	1	1	0	1	0	1	3.5	0
Muskellunge	*Esox masquinongy*	0.4	1	1	0	1	0	1	4	0
Central stoneroller	*Campostoma anomalum*	49.1	2	0	1	0	1	0	2	1
Northern redbelly dace	*Chrosomus eos*	0.0	1	1	0	1	1	0	1	0
Finescale dace	*Chrosomus neogaeus*	0.0	1	1	1	1	1	0	2	0
Redside dace	*Clinostomus elongatus*	49.1	2	0	1	0	1	0	2	1
Lake chub	*Couesius plumbeus*	0.0	1	0	1	1	1	1	3	0
Silver chub	*Macrhybopsis storeriana*	—	2	0	—	1	0	0	1.5	0
Gravel chub	*Erimystax x-punctata*	49.1	5	0	1	0	1	0	2	1
Hornyhead chub	*Nocomis biguttatus*	3.1	1	0	1	0	1	0	2.5	0
River chub	*Nocomis micropogon*	3.1	1	0	1	0	1	0	2.5	0
Golden shiner	*Notemigonus crysoleucas*	21.7	1	1	0	1	0	0	2	0
Pugnose shiner	*Notropis anogenus*	17.0	2	1	0	1	0	0	1.5	0
Emerald shiner	*Notropis atherinoides*	1.5	1	0	1	1	0	0	1	0
Bridle shiner	*Notropis bifrenatus*	0.0	1	1	0	1	1	0	1	0
Striped shiner	*Luxilus chrysocephalus*	0.1	1	1	1	0	1	0	2	0
Common shiner	*Notropis cornutus*	0.1	1	1	1	1	0	0	2	0
Bigmouth shiner	*Notropis dorsalis*	99.6	2	0	0	0	1	0	2	1
Pugnose minnow	*Opsepocodus emiliae*	—	2	1	—	1	1	0	—	0
Blacknose shiner	*Notropis heterolepis*	13.1	1	1	0	1	0	0	1	0
Spottail shiner	*Notropis hudsonius*	0.0	1	1	1	1	1	0	2	0
Silver shiner	*Notropis photogenis*	49.1	2	0	1	0	1	0	2	1
Spotfin shiner	*Notropis spilopterus*	37.9	1	0	1	1	0	0	1	1
Sand shiner	*Notropis stramineus*	0.0	1	1	1	1	1	0	1	1
Redfin shiner	*Notropis umbratilis*	0.0	1	1	1	0	1	0	1	0
Mimic shiner	*Notropis volucellus*	13.1	1	1	0	1	0	0	1	0

Table 2 (*continued*).

Common name	Scientific name	*p* %	Status[a]	FGrass	BGrapeb	FLake	BStream	Piscivor	Agemat	FFlow
Bluntnose minnow	*Pimephales notatus*	0.0	1	1	1	1	1	0	1	0
Fathead minnow	*Pimephales promelas*	0.0	1	0	1	1	1	0	1	0
Blacknose dace	*Rhinichthys atratulus*	49.1	1	0	1	0	1	0	2	1
Longnose dace	*Rhinichthys cataractae*	0.1	1	0	1	1	1	0	2	1
Creek chub	*Semotilus atromaculatus*	0.0	1	0	1	1	1	1	2	0
Fallfish	*Semotilus corporalis*	0.0	1	0	1	1	1	1	2	1
Pearl dace	*Semotilus margarita*	0.0	1	0	1	1	1	0	2	0
Quillback	*Carpiodes cyprinus*	1.5	1	0	0	1	1	0	3	0
Longnose sucker	*Catostomus catostomus*	0.0	1	0	1	1	1	0	6	0
White sucker	*Catostomus commersoni*	0.0	1	0	1	1	1	0	4	0
Northern hog sucker	*Hypentelium nigricans*	63.9	1	0	1	0	1	0	3	1
Silver redhorse	*Moxostoma anisurum*	3.6	1	1	1	1	0	0	8	0
River redhorse	*Moxostoma carinatum*	97.4	2	0	1	0	1	0	8	1
Black redhorse	*Moxostoma duquesni*	76.4	3	0	1	0	1	0	4	1
Golden redhorse	*Moxostoma erythrurum*	7.4	1	0	1	0	1	0	4	0
Copper redhorse	*Moxostoma hubbsi*	100.0	3	0	1	0	0	0	10	0
Shorthead redhorse	*Moxostoma macrolepidotum*	0.0	1	1	1	1	1	0	4.5	0
Greater redhorse	*Moxostoma valenciennesi*	33.0	1	0	1	0	1	0	7	0
Black bullhead	*Ictalurus melas*	0.0	1	1	1	1	1	0	2	0
Yellow bullhead	*Ictalurus natalis*	0.0	1	1	1	1	1	0	2	0
Brown bullhead	*Ictalurus nebulosus*	0.1	1	1	0	1	1	0	3	0
Channel catfish	*Ictalurus punctatus*	15.6	1	0	0	1	0	1	5	0
Stonecat	*Noturus flavus*	0.4	1	0	1	1	0	1	2	1
Tadpole madtom	*Noturus gyrinus*	13.1	1	1	0	1	0	0	1	0
Brindled madtom	*Noturus miurus*	99.3	2	0	0	0	1	0	1	1
America eel	*Anguilla rostrata*	45.7	1	1	0	1	0	1	13	0
Banded killifish	*Fundulus diaphanus*	21.7	1	1	0	1	0	0	2	0
Blackstripe topminnow	*Fundulus notatus*	99.2	2	1	0	0	0	0	1	0
Brook silverside	*Labidisthes sicculus*	0.1	1	1	1	1	0	0	1	0
Burbot	*Lota lota*	0.0	1	0	1	1	0	1	3	0
Atlantic tomcod	*Microgadus tomcod*	7.7	1	0	1	0	0	1	2	0
Brook stickleback	*Culea inconstans*	11.5	1	1	0	0	1	0	1	0
Threespine stickleback	*Gasterosteus aculeatus*	0.0	1	1	0	1	1	1	1	0
Ninespine stickleback	*Pungitius pungitius*	0.0	1	1	0	1	1	1	1	0
Trout-perch	*Percopsis omiscomaycus*	0.0	1	0	1	1	1	0	1	0
White bass	*Morone chrysops*	0.0	1	1	1	1	1	1	3	0
Striped bass	*Morone saxatilis*	99.3	3	0	0	0	0	1	5	0
Rock bass	*Ambloplites rupestris*	0.0	1	0	1	1	1	1	3	0
Pumpkinseed	*Lepomis gibbosus*	0.0	1	1	1	1	1	1	2	0
Bluegill	*Lepomis macrochirus*	0.0	1	1	1	1	1	0	3	0
Longear sunfish	*Lepomis megalotis*	0.2	1	1	1	0	1	0	3	0
Smallmouth bass	*Micropterus dolomieui*	2.6	1	0	1	1	0	1	5	1
Largemouth bass	*Micropterus salmoides*	0.7	1	1	0	1	0	1	5	0
White crappie	*Pomoxis annularis*	0.0	1	1	0	1	1	1	3	0
Black crappie	*Pomoxis nigromaculatus*	0.0	1	1	1	1	0	1	3	0
Yellow perch	*Perca flavescens*	0.0	1	1	1	1	0	1	4	0
Sauger	*Stizostedion canadense*	0.0	1	1	1	1	1	1	4	0
Yellow walleye	*Stizostedion v. vitreum*	1.3	1	1	1	0	0	1	4.5	0
Eastern sand darter	*Ammocrypta pellucida*	99.3	2	0	0	0	1	0	1	1
Greenside darter	*Etheostoma blennioides*	99.3	2	0	0	0	1	0	1	1
Iowa darter	*Etheostoma exile*	13.1	1	1	0	1	0	0	1	0
Fantail darter	*Etheostoma flabellare*	49.1	1	0	1	0	1	0	2	1
Least darter	*Etheostoma microperca*	0.0	1	1	0	1	1	0	1	0
Johnny darter	*Etheostoma nigrum*	0.0	1	0	1	1	1	0	1	0

Table 2 (*concluded*).

Common name	Scientific name	*p* %	Status[a]	FGrass	BGrapeb	FLake	BStream	Piscivor	Agemat	FFlow
Tessellated darter	*Etheostoma olmstedi*	0.0	1	1	1	1	1	0	1	0
Logperch	*Percina caprodes*	25.7	1	0	0	1	1	0	2	1
Channel darter	*Percina copelandi*	95.6	3	0	1	0	0	0	2	0
Blackside darter	*Percina maculata*	3.3	1	1	1	0	1	0	2	1
Freshwater drum	*Aplodinotus grunniens*	2.8	1	0	0	1	1	0	4	0
Eastern mottled sculpin	*Cottus bairdi*	0.2	1	0	1	1	1	0	3	1
Slimy sculpin	*Cottus cognatus*	0.0	1	1	1	1	1	0	3	1
Spoonhead sculpin	*Cottus ricei*	2.7	1	0	1	1	0	0	2	0
Deepwater sculpin	*Myoxocephalus thompsoni*	96.3	2	0	0	1	0	0	4	0
	Discarded species									
Lake herring[b]	*Coregonus artedi*	4.8	1	0	1	1	0	0	3	0
Spring cisco	*Coregonus* sp.	4.8	2	0	1	1	0	0	3	0
Lake whitefish[b]	*Coregonus clupeaformis*	4.8	1	0	1	1	0	0	3	0
Pigmy smelt[b]	*Osmerus spectrum*	2.7	1	0	1	1	0	0	2	0
Grass pickerel[b]	*Esox americanus vermiculatus*	45.7	1	1	0	0	0	1	2	0
Chain pickerel[b]	*Esox niger*	0.2	1	1	0	1	0	1	3	0
Cutlips minnow[b]	*Exoglossum maxillingua*	49.1	1	0	1	0	1	0	2	1
Brassy minnow	*Hybognathus hankinsoni*	19.3	1	1	0	0	1	0	2	0
Eastern silvery minnow[b]	*Hybognathus nuchalis regius*	0.0	1	1	0	1	1	0	2	0
Blackchin shiner[b]	*Notropis heterodon*	—	1	1	—	1	—	0	1	0
Rosyface shiner[b]	*Notropis rubellus*	34.4	1	0	1	0	1	0	1	1
Blue walleye	*Stizostedion vitreum glaucum*	0.1	5	0	1	1	0	1	5	0
Rainbow darter[b]	*Etheostoma caeruleum*	34.4	1	0	1	0	1	0	1	1

Note: FGrass, feeding over grass; BGrapeb, breeding over gravel or pebbles; FLake, feeding in lakes; BStream, breeding in streams; Piscivor, fish eating; Agemat, age at maturity; FFlow, speed of current over feeding area.

[a]1, not at risk; 2–5, at risk (2, vulnerable; 3, threatened; 4, endangered; 5, extinct or extirpated). At risk status is based mostly on COSEWIC evaluations (see text for exceptions).

[b]Status pending at COSEWIC.

The final data set consisted of 117 species from 15 orders and 25 families. The 29 at risk species are from 8 orders and 12 families and the 88 not at risk species are from 14 orders and 23 families. With the exception of the sturgeons and the paddlefish (order Acipenseriformes), both at risk and not at risk species were found within the same orders and families.

Data analysis and comparisons

In this analysis, status (1 = not at risk, 2 = at risk) was the dependent variable, and the life-history traits and ecological data were the independent variables. At risk status includes extinct, extirpated, endangered, threatened and vulnerable species (Table 2).

Life-history traits and ecological data of at risk and not at risk species were compared using a nonparametric univariate test (Npar1way, Wilcoxon scores) and logistic regression. Statistical analysis was conducted using SAS (version 6.03, SAS Institute Inc. 1990). The Wilcoxon score corresponds to the results of Wilcoxon rank-sum test if data are classified in two levels, and to the results of the Kruskal–Wallis test if data are classified in many levels. It was preferred to other linear rank statistics because it is locally most powerful in detecting location shifts in a logistic distribution (SAS Institute Inc. 1990).

The logistic procedure was used with the stepwise option, with the logit link function, and with significance levels for entry into or removal from the model of 0.05. The linear logistic model has the form

$$\text{logit}(p) = \log_e\left(\frac{p}{1-p}\right) = \alpha + \beta\mathbf{x}$$

where p is the response probability of status = 1, α is the intercept parameter, $\mathbf{x}$ is the vector of explanatory variables, and β is the vector of slope parameters. Several models were compared for this data set using their concordance and the log likelihood criterion, to determine which model best defined the differences between the two groups.

Per se, the logistic procedure does not preclude any decision level. Concordance is not measured according to a decision level. Instead, the logistic procedure looks at

Table 3. Probability of being at risk (p %) of some Midwest fish species, including invaders of the Great Lakes – St. Lawrence biozone (according to model 2).

Common name	Scientific name	p %	Status[a]	FGrass	BGrapeb	FLake	BStream	Piscivor	Agemat	FFlow
			At risk species							
Pallid sturgeon	*Scaphyrhynchus albus*	100.0	4	0	1	0	0	0	7	1
Skipjack herring	*Alosa chrysochloris*	99.9	1	0	0	0	0	1	2	1
Speckled chub	*Hybopsis a. tetranemus*	99.8	2	0	1	0	0	0	1	1
Sturgeon chub	*Hybopsis gelida*	99.8	2	0	1	0	0	0	1	1
Sicklefin chub	*Hybobsis meeki*	99.8	2	0	1	0	0	0	1	1
Pallid shiner	*Notropis amnis*	99.2	1	1	0	0	0	0	1	0
Blue sucker	*Cycleptus elongatus*	100.0	2	0	1	0	0	0	4	1
Crystal darter	*Ammocrypta asperalla*	100.0	2	0	0	0	0	0	1	1
Arkansas darter	*Etheostoma cragini*	0.0	2	1	1	0	1	0	1	0
Niangua darter	*Etheostoma nianguae*	1.3	3	0	1	0	1	0	1	0
Blustripe darter	*Percina cymatotaenia*	0.1	2	1	1	0	1	0	2	0
Longnose darter	*Percina nasuta*	49.1	3	0	1	0	1	0	2	1
Stargazing darter	*Percina uranidea*	49.1	2	0	1	0	1	0	2	1
Reclassification success 76.9%										
			Not at risk species							
Chestnut lamprey	*Ichthyomyzon castaneus*	43.6	1	1	0	0	1	1	7	1
Shortnose gar	*Lepisosteus platostomus*	5.2	1	0	0	1	0	1	3	0
Ghost shiner[b]	*Notropis buchanani*	99.5	1	1	0	0	0	0	2	0
Red shiner	*Notropis lutrensis*	0.0	1	1	1	1	1	0	1	0
Lake chubsucker[b]	*Erimyzon sucetta*	0.1	1	1	0	1	1	0	3	0
Bigmouth buffalo[b]	*Ictiobus cyprinellus*	2.1	1	0	0	1	1	0	3.5	0
Black buffalo[b]	*Ictiobus niger*	94.4	1	0	0	0	1	0	3.5	0
Spotted sucker[b]	*Minytrema melanops*	0.0	1	1	1	1	1	0	4	0
Flathead catfish[b]	*Pylodictis olivaris*	9.1	1	0	0	1	0	1	4	0
Starhead topminnow	*Fundulus notti*	21.7	1	1	0	1	0	0	2	0
Green sunfish[b]	*Lepomis cyanellus*	0.0	1	1	1	1	1	1	2	0
Warmouth[b]	*Lepomis gulosus*	0.0	1	1	1	1	1	0	2	0
Orangespotted sunfish[b]	*Lepomis humilis*	0.0	1	0	1	1	1	0	2	0
Banded darter	*Etheostoma zonale*	99.6	1	0	0	0	1	0	2	1
Grass carp[b]	*Ctenopharyngodon idella*	na	na	na	na	na	na	na	na	na
Reclassification success	78.6%									
Total reclassification success	77.8%									

Note: Species with a p value larger than 49% are considered to be at risk. FGrass, feeding over grass; BGrapeb, breeding over gravel or pebbles; FLake, feeding in lakes; BStream, breeding in streams; Piscivor, fish eating; Agemat, age at maturity; FFlow, speed of current over feeding area; na, not applicable.

[a]Status is based on AFS evaluations (Williams et al. 1989). 1, not at risk; 2–5, at risk (2, special concern; 3, threatened; 4, endangered; 5, extinct).
[b]Invader of the Great Lakes – St. Lawrence biozone.

all possible pairs of at risk – not at risk values (29·88 = 2552 pairs in our case) and counts the concordant pairs, i.e., pairs where the p value for the species at risk is larger than the p value for the species not at risk (if it is smaller, the pair is said to be discordant, if values are equal, it is a tie). Any decision level can therefore be used depending upon how one weighs species at risk of extinction.

To assess the applicability of the model generated by the logistic regression, comparisons were made with a subset of 27 different fish species from the adjacent Midwest states of the U.S.A. This area is part of the Prairie biozone and of the Gulf of Mexico drainage basin. The Midwest data set included 13 species at risk and 14 species not at risk (including all but one of the invaders previously excluded from our analysis) (Table 3). The species at risk were defined using AFS status (Williams et al. 1989), except for the skipjack herring (*Alosa chrysochloris*) and the pallid shiner (*Notropis amnis*), which were considered to be at risk for the analysis because they may be heading toward extirpation in the Midwest. According to Becker (1983), the

Table 4. Hierarchy of models derived by logistic regression (see Table 1 for the meaning of the codes).

Model	Equation	Concordance (%)	p	−2LogL
1	Logit (p) = 4.46 − 4.06 Boreal + 0.12 Lifespan − 3.97 FGrass − 4.86 BGrapeb − 3.55 FLake	96.6	0.0001	40.5
2	Logit (p) = 7.52 − 3.34 FGrass − 5.65 BGrapeb − 6.67 FLake − 6.82 BStream − 5.57 Piscivor + 0.61 Agemat + 3.71 FFlow	97.1	0.0001	38.9
3	Logit (p) = 8.45 − 6.14 FLake − 5.51 FGrass − 3.82 BGrapeb − 4.97 BStream + 5.27 FSea	92.3	0.0001	51.9
4	Logit (p) = 5.08 − 3.51 FLake − 3.73 FGrass − 3.33 BGrapeb − 2.44 BStream	89.1	0.0001	66.1

Note: Logit (p) is the logit link function between the vector of explanatory variables and the vulnerability probability p (logit $p = \log(p/1 - p) = \alpha + \beta x$.

skipjack herring is rare in South Dakota, threatened in Iowa, and extirpated in Wisconsin, while the pallid shiner is threatened in Wisconsin, is endangered in Illinois, and has not been seen in Missouri since 1956. The ghost shiner (*Notropis buchanani*) has become rare and may be extirpated above pool 14 of the Mississippi River (Becker 1983). However, since this species is an invader in the Great Lakes – St. Lawrence biozone and is not considered to be at risk by AFS, it was considered not at risk in this analysis. Finally, the grass carp (*Ctenopharyngodon idella*) is an invader in the Great Lakes – St. Lawrence biozone but it originates from Asia. It was therefore excluded from our analysis.

Results

The model

The nonparametric univariate analysis of the 49 life-history traits and ecological data indicated that 14 life-history traits and ecological factors were significantly different between species at risk and those not at risk (prob |Z| ≤0.05 or prob CHISQ ≤0.05). Traits associated with being at risk were moderate to fast flow in feeding area (FFlow) and breeding in deep waters (BDepth). Those associated with not being at risk were presence in the Boreal biozone (Boreal), fish-eating feeding regime (Piscivor), feeding in lakes (FLake), feeding in ponds (FPond), feeding in rivers (FRiver), feeding over grasses (FGrass), breeding over gravel or pebbles (BGrapeb), breeding in lakes (BLake), breeding in ponds (BPond), breeding in streams (BStream), nesting (Nest), and parental care (Parcare) (Table 1).

We used logistic regression to develop a hierarchy of models. The most general model (model 1, Table 4) included presence in the Boreal biozone as the most important independent variable. This separates species with a more northerly distribution from those with a more southerly distribution. Other variables selected by the procedure were, in order of strongest contribution (Table 4), age at maturation (Agemat), breeding over gravel or pebbles, speed of water flow in feeding area, and fish-eating feeding regime.

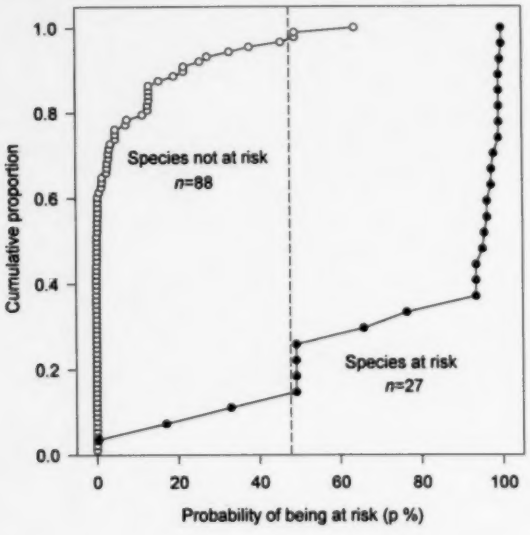
The next model we developed was less general: it included all testable factors but excluded any reference to geographic range (model 2, Table 4). Thus, there were no variables tested in this model that overlapped those used by COSEWIC to determine status. Model 2 gave the highest concordance (97.1%) and the best likelihood (38.9). The variables selected were, in order of strongest contribution, feeding over grasses, breeding over gravel or pebbles, feeding in lakes, breeding in streams, fish-eating feeding regime, age at maturation, and speed of water flow in feeding area. This model exhibited the best fit for this data set and was therefore used to assess the probability of extinction of all fish species.

We then attempted to determine the influence of each variable in model 2. This process allowed us to create models with variables that were fewer in number and easier to estimate. When age at maturation was removed from the analysis, the procedure dropped the feeding regime and the speed of water flow in feeding area, and replaced these factors by feeding in estuaries and oceans (FSea). The resulting model (model 3, Table 4) had a very high concordance (92.3%) and a good likelihood (−2 log likelihood = 51.9). Finally, when FSea was removed, the procedure produced a model with only four factors, still 89.1% concordant with a likelihood of 66.1 (model 4, Table 4).

Model 2 was used to calculate the probability of at risk values (p), from $p = e^{\text{logit}(p)}/(1 + e^{\text{logit}(p)})$, for all species (Table 2). The distribution of the p values for at risk and not at risk species is given in Fig. 2. The probability of being at risk increases with p. Although the logistic procedure does not have a set decision level, people usually use logit(p) = 0 (p = 50%) as the decision level for the reclassification of data. With model 2, this decision level produced a large reclassification error (25.9%) for at risk species, mainly because there were 4 values at 49.1%. We chose instead p = 49% as the decision level. At this level the reclassification error was only 5.2%: 3.4% of the species not at risk were predicted to be at risk by the model, and 11.1% of the species at risk were predicted to be not at risk. The mean p value was 79.3% for species at risk and 6.3% for species not at risk. The resulting high mean p ratio of 12.6 indicates that the model was both a good predictor of status and able to distinguish between these fish species on the basis of their status.

Owing to insufficient data, two at risk species were not included in the model. They were the silver chub (*Macrhybopsis storeriana*) and the pugnose minnow (*Opsepocodus emiliae*). The former fitted the model if it did not breed over gravel or pebbles whereas the latter, regardless of its breeding substrate and its age at maturation, did not fit the model.

Fig. 2. Cumulative proportion of fish species at risk and not at risk in relation to estimated probability of being at risk (p %) by model 2. Species to the right of the decision level (p = 49%, broken line) are predicted to be at risk of extinction by the model.



Assessment of model applicability

When model 2 was applied to a set of Midwest fish species with the same decision level of p = 49%, it predicted correctly whether a species was at risk or not in 77.8% of the cases (Table 3). The reclassification success for this data set was 76.9% for species at risk and 78.6% for species not at risk. This latter group included mostly invaders of the Great Lakes – St. Lawrence biozone. As expected, all but two invading species had a p value below 49%. One exception, the ghost shiner (p = 99.5%), is probably highly at risk in the Midwest states (Becker 1983).

Discussion

We believe that the confounding effects of taxonomy in the analyses are minimal, if any, because all species were included in the analysis, thus precluding taxonomic bias. Moreover, the species comprised a highly diverse taxonomic group with no family or genus overrepresented, and species at risk and species not at risk are both spread across the entire spectrum of taxonomic groups.

Vulnerability profile

Fish species at risk and not at risk in the Great Lakes – St. Lawrence biozone significantly differ by their life-history traits and ecological data. The results of the logistic regression show that the following characteristics increase the probability of a species being at risk: late maturing (K selected), feeding not over grass but in moderate to strong currents, not piscivorous, not inhabiting lakes, and not breeding in streams or over gravel or pebble beds. A species having this profile and maturing at 6 years of age or more has a 100% chance of being at risk. No fish species in the data set has such a profile. The closest is the copper redhorse (*Moxostoma hubbsi*; p = 100%), which is threatened. The Wilcoxon scores also indicate that breeding in deep waters (over 30 m) increases the risk of extinction. Good examples of deepwater breeders at risk of extinction are the Great Lakes ciscos and the deepwater sculpin (*Myoxocephalus thompsoni*).

At the opposite end of the spectrum, the results of the logistic regression show that the following profile reduces the probability of a species being at risk: maturing early (r-selected species), being a piscivore, living in lakes over grass, feeding in slow-moving or still waters, and breeding in streams over gravel or pebbles. A species having this profile and maturing at 2 years of age has a 0% chance of being at risk. The pumpkinseed (*Lepomis gibbosus*) has that exact profile and several other species are close to it (Table 2). The Wilcoxon scores add the following factors to the profile: feeding in ponds or rivers, breeding in lakes or ponds, nesting, and parental caring. These factors say the same thing as the logistic regression but in a different way: species living in ponds do live in fact in slow-moving or still waters, and most species nesting and exhibiting parental care do breed over gravel or pebbles.

The vulnerability profile suggests that human activity primarily affects species living in clear moving waters. These species are less tolerant of changes in water quality, e.g., turbidity, than those living in ponds, lakes, or rivers. However, this being the case, why are species that breed in the same clear moving water (BStream) over gravel or pebbles (BGrapeb) not at risk? The answer may lie in the numerous flood-control works that are found in the Great Lakes – St. Lawrence biozone. On the one hand, flood-control schemes frequently destroy backwaters and other areas of slack water, which are important spawning areas for species breeding over grass, mud, or silt (Wotton 1992). On the other hand, the tail waters at the base of the dams often provide turbulent, oxygen-rich water and a substrate suitable for gravel-spawning fish (Neilson et al. 1986).

The predictions

Of the five predictions suggested by the hypothesis at the beginning of this paper, two were supported, one was rejected, and two could not be determined.

Life-history variables associated with at risk status reflect the cultural stresses of the fish fauna of the Great Lakes – St. Lawrence biozone. Lakes, being a safe place to live, reflect the reduction and destruction of other habitats through farming, urbanization, industrialization, and damming. The same reasoning likely prevails for breeding habitat: one must remember that midwater lake spawners, mostly coregonids, have been overfished and affected by the introduction of the marine lamprey.

Since it includes age at maturation, our model is coherent with the concept of the r–K continuum (Regier 1979). Our results also partly support the life-history theory (Stearns 1976), which states that in unstable environments, species with late maturity, multiple broods, a few, large young, parental care, and low fecundity should be more prone to extinction. Parental care, which Stearns (1976) considered a K-factor but does not appear now to be characteristic of r- and K-strategies (Partridge and Harvey

1988), was found to put species not at risk in the Great Lakes – St. Lawrence biozone. Almost all species exhibiting parental care are usually considered r-selected species. They are small catfishes (*Ictalurus* sp., *Noturus* sp.), sticklebacks (*Gasterosteus* sp.), and sunfishes (*Lepomis* sp., *Pomoxis* sp.). Four other K-factors were not found to be significant by the Wilcoxon score: length, lifespan, clutch size, and number of broods (Table 1).

Contrary to our prediction, we concluded that coldwater species were less at risk than warmwater ones. The Boreal variable, indicative of coldwater species, entered model 1 with a minus sign. This meant that species living in both the Great Lakes – St. Lawrence and the Boreal biozones, therefore coldwater ones, had more chances of not being at risk than southern warmwater species. This suggests that warmwater invaders are competing with warmwater species of the Great Lakes – St. Lawrence biozone.

As for migratory behavior, we were surprised that no link could be established with vulnerability. The Wilcoxon score for this variable (0.146, Table 1) was low, but still not sufficiently significant to enter the model. The explanation may lie in the highly diverse degree of potamodromy exhibited by many fish species, and the facultative migration in some not at risk species in the Great Lakes, such as alewife (*Alosa pseudoharengus*) and sea lamprey.

Finally, the fact that being a piscivore placed a species in the not at risk category reflected possibly important changes in the food webs, e.g., a decline in mollusks. We were unable to show that species with specialized feeding regimes were more at risk than others, but this may only have reflected the lack of refinement in the feeding levels used to build the model. The role of Piscivor in the model also suggests that top-level species are less at risk than intermediate or bottom ones, which follows Mandrak's (1989) suggestion. This should have been confirmed by the type of predator (Predator), but the Wilcoxon score was not significant for this factor (Table 1). Since Predator had only two levels, we suggest that it could not account for all of the food web levels present in the Great Lakes – St. Lawrence biozone.

The model presented here is empirical. It does not try to explain why some life-history traits or ecological data put fish species from the Great Lakes – St. Lawrence biozone more at risk than others. However, the results obtained suggest a hypothesis as to the possible cause of vulnerability of fish species in the Great Lakes – St. Lawrence biozone: vulnerability is linked to general feeding and breeding habits rather than to ecological specialization. Generalist species may be more vulnerable than specialized ones.

The models

The nonparametric univariate results indicated differences in life-history traits and ecological data between species at risk and not at risk. This first analysis enabled us to proceed with the logistic regression knowing that there were many variables that would distinguish between at risk and not at risk species.

Preliminary analysis using logistic regression had shown that Canadian range alone, expressed as the number of provinces in which the species is found, was 83% concordant with status. Since vulnerability status is often defined by COSEWIC on the basis of restricted range, any variable that included range was excluded from our final model (model 2).

We compared the decision level inherent to the logistic regression ($p = 50\%$) with various other levels. We decided to use $p = 49\%$ as our decision level because it minimized the reclassification error to 5.2% and weighed at risk species only slightly more than not at risk species. Figure 2 shows that sliding the p value of the decision level further to the left does not change this error greatly. The choice of the decision level depends on how at risk species are weighed against those not at risk. One may decide to be very conservative and use a decision level of 30%: the reclassification error would then go up to 7.0%, and chances of classifying a species not at risk when it was would decrease to 7.4%. Conversely, one might decide to be tolerant and use a decision level of 60%: the reclassification error would again increase to 7.0%, but the chances of classifying a species at risk when it was not would decrease to only 1.1%.

The strength of the variables in the final model (model 2) was assessed from the concordance each variable or set of variables gave to the model. Speed of water flow in feeding area, feeding regime, and age at maturation added little concordance to the model. The difference between the concordance value with and without these three variables present was 8%. Feeding habitat, feeding substrate, breeding habitat, and breeding substrate provided 89% concordance. When removed, age at maturation was replaced by FSea for a loss of concordance of 5%, and a slightly reduced likelihood. FSea is much easier to estimate than age at maturation, which is the only continuous variable in the model. However, the nonparametric analysis (Table 1) showed that this factor is not significantly different between species at risk and those not at risk. Model 4 is therefore more meaningful than model 3. Importantly, model 2 indicates that, given the anthropocentric stresses of the biozone, any fish species maturing at 18 years or more is at risk. Sturgeons are included in this category.

Two factors of model 2 were found to directly put a species at risk (negative parameter estimate): age at maturation and speed of water flow in feeding areas. The weakness of such a model is that an error in the value of one predictor greatly affects the results. For example, an error in feeding flow changes the p value from 2.7 to 52.8%. Another difficult estimator is fish feeding regime. Since both feeding flow and fish feeding regime are not easy to estimate and give room for wide interpretation, one may instead use the reduced forms of model 2, i.e., models 3 or 4 (Table 4). Other factors could not be removed from the model without a great loss of concordance and likelihood.

In examining the p values calculated from our model (model 2), we found that one not at risk species was predicted to be at risk by the model, and a second was borderline. The northern hog sucker (*Hypentelium nigricans*) produced a high p value (63.9%) as though it was at risk (Table 2). This suggests that perhaps the status of that species should be examined carefully. Also, the American eel (*Anguilla rostrata*) has a borderline p value of 45.7%. This species may not appear to be at risk because of its

wide geographic range, but it may in fact be in trouble. The American eel, a late-maturing species, may be more at risk than is presently thought because it is heavily contaminated by chemicals and there appears to have been a drastic decline in recruitment in Lake Ontario between 1985 and 1992 (Castonguay et al. 1994).

As expected, the model could not predict the extinction of the blue walleye nor the vulnerability of the spring cisco. However, it correctly detected all extirpated or extinct species in the Great Lakes – St. Lawrence biozone, including the borderline gravel chub (p = 49.1%).

General applicability

Model 2 is specific to the Great Lakes – St. Lawrence biozone, but it is also applicable to the adjacent Midwest area, since reclassification success in this other data set is 77.8%. When applied to the Midwest data set, model 2 found that all but two species invading the Great Lakes – St. Lawrence biozone are at low risk of extinction. This corresponds well with the profile of an invader species as defined by Mandrak (1989): r-selected species, neither predator nor prey, and nonguarding lithophil (rock or gravel) or phytophil (plant) wetland spawner.

However, it is doubtful that model 2 could be used in the southern United States where fishes occur in very different habitats and where invasion by exotic fish species is a major threat (Williams et al. 1989). To further test the model, one could look at the fish fauna of central or northern Europe where habitats and anthropocentric stresses resemble those of the Great Lakes – St. Lawrence biozone.

We used model 2 to predict the status of the native species originally discarded from the analysis (Table 2). The model predicted that no discarded species is clearly at risk. The blackchin shiner (*Notropis heterodon*), for which a vulnerable status is proposed (Campbell 1993), is not at risk according to the model, even if it breeds in at risk conditions, i.e., not in streams nor over pebbles or gravel. However, two species, the grass pickerel (p = 45.7%) and the cutlips minnow (*Exoglossum maxillingua* p = 49.1%), are borderline, and should be examined closely.

We believe that this modeling technique can have a wide and useful application. It is ideal for use as a guideline for classifying the status of species and can be used to determine which species should be singled out for conservation. Also, it could easily be used to complement decisions regarding conservation and status of the species. At the moment, for lack of better tools, most status decisions are based on the degree of range restriction and population depletion. The model presented here has proven useful in predicting the vulnerability of species with wide geographic ranges, such as the Atlantic sturgeon and the American eel.

The model also has heuristic value in supporting the role of certain biological characteristics, generally associated with K-selected species life-history traits, in the determination or prediction of a species' vulnerability. Furthermore, a similar modeling approach could be used profitably with other taxonomic groups for which appropriate life-history data exist. Mammals, birds, reptiles, and amphibians for which life histories are well known would easily lend themselves to similar analyses.

Acknowledgments

We thank Isabelle Tartier for cross-checking the data, Antoine Morin for statistical assistance, Lenore Fahrig, Scott Findlay, and Daniel Gagnon for reviewing the manuscript.

References

Beaulieu, H. 1993. Liste des espèces de la faune vertébrée susceptibles d'être désignées menacées ou vulnérables. Ministère du Loisir, de la Chasse et de la Pêche, Quebec.

Becker, G.C. 1983. Fishes of Wisconsin. The University of Wisconsin Press, Madison, Wis.

Begon, M., J.L. Harper, and C.R. Townsend. 1986. Ecology: individuals, populations and communities. Blackwell, Oxford.

Burnett, J.A., T.C. Dauphiné, S.H. McCrindle, and T. Mosquin. 1989. On the brink: endangered species in Canada. Western Producer Prairie Books, Saskatoon, Sask.

Campbell, R.R. 1988. Rare and endangered fishes and marine mammals of Canada. COSEWIC Fish and Marine Mammal Subcommittee Report No. IV. Can. Field-Nat. 102: 81–86.

Campbell, R.R. 1993. Rare and endangered fishes and marine mammals of Canada. COSEWIC Fish and Marine Mammal Subcommittee Status Report No. IX. Can. Field-Nat. 107: 395–401.

Castonguay, M., P.V. Hodson, C.M. Couillard, M.J. Eckersley, J.-D. Dutil, and G. Verreault. 1994. Why is recruitment of the American eel, *Anguilla rostrata*, declining in the St. Lawrence River and Gulf? Can. J. Fish. Aquat. Sci. 51: 479–488.

Francis, G.R., J.J. Magnuson, H.A. Regier, and D.R. Talhelm. 1979. Rehabilitating Great Lakes ecosystems. Great Lakes Fish. Comm. Tech. Rep. No. 37.

Lande, R. 1988. Genetics and demography in biological conservation. Science (Washington, D.C.), 241: 1455–1460.

Lee, D.S., C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister, and J.R.J. Stauffer. 1980. Atlas of North American freshwater fishes. North Carolina State Museum of Natural History, Raleigh, N.C.

Mandrak, N.E. 1989. Potential invasion of the Great Lakes by fish species associated with climatic warming. J. Great Lakes Res. 15: 306–316.

McAllister, D.E., B.J. Parker, and P.M. McKee. 1985. Rare, endangered and extinct fishes in Canada. Syllogeus Series No. 54. National Museums of Canada, Ottawa, Ont.

Miller, R.R., J.D. Williams, and J.E. Williams. 1989. Extinctions of North American fishes during the past century. Fisheries, 14: 22–38.

Neilson, J.D., R.J. Sheenan, and D.J. Orth. 1986. Impacts of navigation on riverine fish production in the United States. Pol. Arch. Hydrobiol. 33: 277–294.

Paloheimo, J.E., and H.A. Regier. 1982. Ecological approaches to stressed multispecies fisheries resources. *In* Multispecies approaches to fisheries management advice. *Edited by* M.C. Mercer. Can. Fish. Aquat. Sci. Spec. Publ. No. 59. pp. 127–132.

Partridge, L., and P.H. Harvey. 1988. The ecological context of life history evolution. Science (Washington, D.C.), 241: 1449–1455.

Regier, W.L. 1979. Changes in species composition of Great Lakes fish communities caused by Man. Trans. N. Am. Wildl. Nat. Resour. Conf. 44: 550–556.

SAS Institute Inc. 1990. SAS Technical Report No. P-200. SAS/STAT software: CALIS and LOGISTIC procedures, release 6.04. SAS Institute Inc., Cary, N.C.

Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Stearns, S.C. 1976. Life-history tactics: a review of the ideas. Q. Rev. Biol. 51: 3–47.

Williams, J.E., J.E. Johnson, D.A. Hendrickson, S. Contreras-Balderas, J.D. Williams, M. Navarro-Mendoza, D.E. McAllister, and J.E. Deacon. 1989. Fishes of North America endangered, threatened, or of special concern: 1989. Fisheries, 14: 2–20.

Winemiller, K.O., and K.A. Rose. 1992. Patterns of life-history diversification in North American fishes: implications for population regulation. Can. J. Fish. Aquat. Sci. 49: 2196–2218.

Wotton, R.J. 1992. Fish ecology. Chapman and Hall, New York.

Implications of increased UV-B induced photoreduction: iron(II) enrichment stimulates picocyanobacterial growth and the microbial food web in clear-water acidic Canadian Shield lakes

J.C. Auclair

Abstract: Ozone depletion and associated increases in UV-B radiation could increase the photoreduction of iron in Canadian Shield lakes of the boreal forest zone. Since photoreduced iron(II) is more soluble than iron(III), and the reoxidation rate slower in acidic (pH = 5–6) lakes, phytoplanktonic growth and (or) species composition may be altered where iron is growth limiting. This hypothesis was tested by amending herbivore-free lake-water enclosures with phosphorus, phosphorus and iron(II), or phosphorus, ammonium, and iron(II) in lac Tantaré, a clear-water acidic lake in Quebec. In herbivore-free unenriched control enclosures, phytoplanktonic abundance doubled within 48 h, demonstrating unequivocally that herbivore grazing (top-down control) intensity controls phytoplanktonic abundance in early spring in this lake. However, at chlorophyll *a* concentrations 2–3 times the ambient lake levels, picocyanobacterial production and abundance were greater in the phosphorus and iron enclosures, relative to the phosphorus-only enclosures. This resulted in enhanced growth of the indigenous chrysophytes presumably through increased mixotrophy. In spite of a 10-fold increase in nanoflagellate abundance, we were unable to discern any strong competitive interactions among the mixotrophic chrysophytes. This strongly suggests that most if not all of their iron quotas are obtained from their picoplanktonic prey.

Résumé : L'amincissement de la couche d'ozone et l'accroissement de la radiation UV-B pourraient augmenter la photoréduction du fer des eaux du Bouclier de l'écozone boréale. Puisque le Fe(II) photoréduit est plus soluble que le Fe(III), et la réoxydation plus lente en milieu acide (pH = 5–6), la croissance phytoplanctonique et (ou) la composition en espèces pourraient être altérées dans les milieux où le fer limite la croissance. Cette hypothèse a été vérifiée par enrichissement d'enclos d'eau de lac (libres d'herbivores) avec soit, du phosphore, du phosphore et du Fe(II) ou du phosphore, ammonium et Fe(II) au lac Tantaré, un lac acide à forte transparence au Québec. Au printemps, dans les enclos témoins, l'abondance phytoplanctonique a doublé sur une période de 48 h démontrant l'étroit contrôle de l'abondance par les brouteurs herbivores. Cependant, lorsque les concentrations en chlorophylle *a* atteignent 2–3 fois les valeurs observées dans le lac, la production et l'abondance des picocyanobactéries ont été supérieures dans les enclos enrichis avec du phosphore et du fer, par rapport à ceux enrichis avec du phosphore seulement. Cette croissance a contribué à un accroissement des espèces chrysophycées indigènes présumément par une augmentation de la mixotrophie. Bien qu'il y ait eu une augmentation de l'abondance des nanoflagéllés, nous n'avons pas discerné une forte compétition entre les chrysophycées mixotrophes. Cette observation suggère fortement que la majorité du quota en fer cellulaire fut obtenu à partir de leurs proies picoplanctoniques.

Introduction

The discovery that iron enhances phytoplanktonic growth in shipboard incubations of high nutrient low-chlorophyll seawater from several regions of the world's oceans (Martin and Fitzwater 1988) has stimulated interest in the biological role of this element in oceanography. Early studies of freshwater lakes (Saunders 1957; Shapiro 1957) considered that the relatively large pool of dissolved organic

Received July 28, 1994. Accepted March 3, 1995.
J12508

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matter comprising humic and fulvic acid effectively chelated iron and other micronutrients, maintaining their availability to phytoplankton. Iron was not considered to be potentially limiting to phytoplanktonic growth in lakes. Subsequent studies demonstrated that chelated iron additions to marl lakes containing low amounts of dissolved organic material greatly increased primary production (Schelske et al. 1962), and iron limitation as a result of high demand during intense periods of nitrogen fixation has been documented in eutrophic hardwater lakes (Murphy et al. 1983; Wurtsbaugh and Horne 1983).

Working with weakly buffered and poorly mineralized Canadian Shield lake waters and using a bioassay approach, Sakamoto (1971) showed that iron concentration and (or) chelators limit ^{14}C uptake in laboratory-incubated lake-water samples. Subsequently, Jackson and Hecky (1980) found significant inverse correlations between primary productivity (i.e., light-saturated rates of photosynthesis measured in an incubator), dissolved organic material, and undialysable colloidal iron in humic impoundments on the Canadian Shield. On this basis, as well as on the basis of poor relationships among productivity, light, and phosphate, they suggested that iron might be tightly bound to dissolved humic material and thus unavailable to phytoplankton. Owing to the low solubility of Fe^{3+} ($K_{sp} = 10^{-20}$) and complex aqueous chemistry, iron may be kinetically limiting to phytoplanktonic growth (Rich and Morel 1990), perhaps resulting in competition for this microelement among organisms constituting the freshwater microbial community as suggested earlier (Murphy et al. 1976).

An elegant study by Finden et al. (1984) revealed the unexpected importance of light-induced reduction of fresh natural iron(III) oxides and iron associated with humic substances in supporting phytoplanktonic growth. In the marine environment, the photoreductive cycle of reduction, oxidation, and reprecipitation of amorphous Fe solids, their chemical lability, and their bioavailability (cf. Wells et al. 1991*a*) are now well characterized (Wells and Mayer 1991; Wells et al. 1991*b*).

In contrast, except for studies investigating iron–phosphorus relationships in highly humic systems (Francko and Heath 1982), the role of iron photoreduction and its effect on planktonic dynamics have not been investigated in freshwaters. Given that increases in atmospheric UV-B-radiation as a result of ozone depletion should increase photoreductive processes occurring at lake surfaces, iron(II) concentrations should increase in clear-water acidic lakes given the low reoxidation rate of Fe(II) to Fe(III). Experimental studies have revealed that in situ rates of photoreduction in the spring and early summer are not substrate limited (Aubé 1994; J.C. Auclair, unpublished results).

Because the far more soluble photoreduced iron (Fe(II): ferrozine labile) appears to be assimilated almost as efficiently as Fe(III) in laboratory culture work (Anderson and Morel 1980; Schenck et al. 1988), planktonic growth and species composition might be affected by increased iron availability, particularly in clear-water acidic lakes.

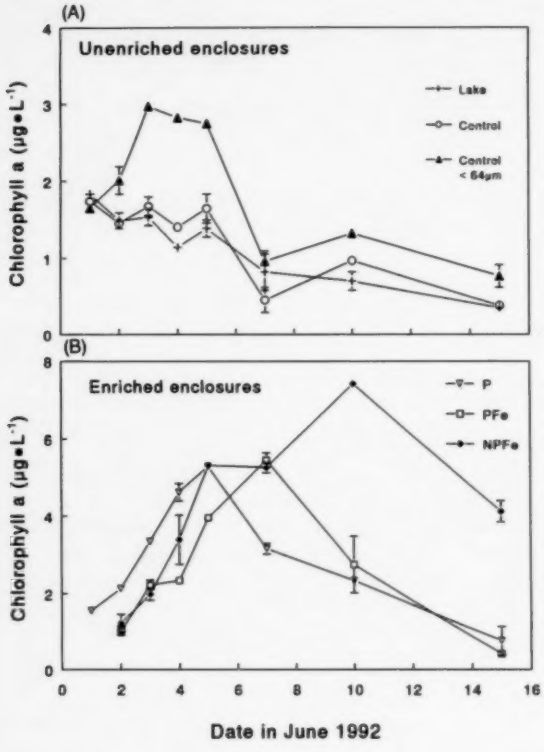
This hypothesis was tested by directly enriching lake-water enclosures with nitrogen, phosphorus, and Fe(II) in lac Tantaré (47°04′N, 71°34′W), an oligotrophic clear-water acidic lake located 40 km north of Quebec City. Using herbivore-free enclosures, a differential enrichment scheme (N, P, and Fe) was employed to examine the role of iron with respect to changes in an early-spring phytoplanktonic community, specifically temporal changes in growth, abundance, and species composition. This enrichment approach also enabled the determination of the threshold levels of phytoplanktonic abundance required for iron chemistry to begin influencing community structure.

Materials and methods

In assessing possible iron limitation to plankton in acidic lakes there are several advantages in using Fe(II) enrichments as opposed to Fe(III). Fe(II) is more soluble, and at the low lake pH (5.3–5.6) the rate of reoxidation of Fe(II) to Fe(III) is reduced. In turn, Fe(III) exchange and hydrolytic dissolution of natural amorphous iron colloids, which might result in the displacement of adsorbed toxic trace metals and complicate experimental interpretation, is minimized. The low concentration of dissolved organic carbon (2.28 ± 0.30 mg·L^{-1}, $n = 4$) in the study lake also reduces the possible complexation of iron by natural organic ligands.

The enrichment experiments were performed in ~ 570-L polyethylene lake-water enclosures in an unstratified basin ($Z_{max} = 4$ m) of the lake. Linear polyethylene tubing (UV-$B_{trans} > 87\%$, 60 cm diam.) was cut and sealed at one end to obtain 2-m tubes. Prior to the experiment, the tubes were rinsed three times with lake water, filled, closed, and left to precondition at the lake surface for a minimum of 6 h. The enclosures were then emptied, and during final filling, crustacean herbivores and rotifers were excluded by attaching the tube onto the bridle of a new 64-μm standard oceanographic plankton net. To attach the tube and net, the polyethylene material was folded over the bridle and ring and firmly held in place by plastic clips. This net–tube assembly was then gently horizontally towed by hand (1–2 cm·s^{-1}), approximately 0.5 m below the surface, by allowing the boat to drift downwind from an anchored raft. When filling was complete, the net was withdrawn and the enclosures were attached to a raft constructed entirely from acrylonitrile butadiene styrene (ABS) and chlorinated polyvinyl chloride (CPVC) plastic plumbing; enclosed water contacted polyethylene surfaces only. A total of eight herbivore-free enclosures were installed, consisting of duplicate controls (Ctrl < 65 μm), phosphorus-enriched enclosures (P), enclosures enriched with phosphorus and iron (PFe), and enclosures enriched with nitrogen (as NH_4), phosphorus, and iron (NPFe). To monitor the effect of grazing in a confined environment we also installed duplicate enclosures with intact unfiltered lake water (Ctrl). Unfiltered and filtered control and P-enriched enclosures were filled in the late afternoon on June 1, 1992, while PFe and NPFe enclosures were filled and enriched within 12 h of the first set, in the early morning on June 2, 1992. Meteorological forcing was minimal during enclosure installation; the sky was overcast with light rain and calm wind on these 2 days. Thus, with respect to the physiological status of the planktonic populations, there is no reason to expect different initial conditions between the two sets of enclosures.

Fig. 1. (A) Average and range (error bars) of chlorophyll *a* concentrations ($\mu g \cdot L^{-1}$) measured in duplicate lake-water (Lake), unfiltered control, and filtered control, (Control <65 μm) nonenriched enclosures over the experimental period. (B) Same measurements in duplicate phosphorus (P), phosphorus and iron (PFe), and ammonium, phosphorus, and iron (NPFe) enriched enclosures. Error bars within point size are not shown.



Filtered and unfiltered enclosures were enriched by injecting 5 mL of concentrated stock solutions through a length of CPVC plastic pipe with compressed air from a hand-operated plastic pump. I added phosphorus and nitrogen to give final enclosure concentrations of approximately 3 μM P-PO_4 as KH_2PO_4 and 60 μM N-NH_4 as NH_4Cl. These high nutrient concentrations were used to prolong the spring growth period, as well as to provide a large excess of N and P relative to Fe, to determine the threshold phytoplanktonic abundance required for iron chemistry to begin influencing species composition. Iron was added as $FeCl_2 \cdot 4H_2O$ to obtain final concentrations of 0.2 μM Fe(II), or about 2–3 times the late-summer ambient levels in the lake. Daily integrated water samples (0.25 L) from each enclosure and the lake were obtained using a 2 m length of CPVC pipe. Temperature and pH were measured in situ electrometrically. Lake optical extinction coefficients within the photosynthetic (PAR; 400–700 nm) and UV (300–400 nm) ranges were obtained using a LICOR spectroradiometer.

Table 1. Phytoplankton exponential growth rates determined from initial increases in chlorophyll *a* concentrations in unenriched and enriched herbivore-free enclosures.

Enclosure	Growth rate (μ, day^{-1})	Interval (days)	n	r^2
Ctrl < 65 μm	0.64±0.027*	1–3	6	0.99
P	0.87±0.047	1–3	6	0.99
PFe	0.93±0.068	2–5	8	0.97
NPFe	1.37±0.12	2–5	8	0.96

Note: Growth rate values are given as the mean ± SE.
*$p < 0.05$.

Phytoplanktonic growth was followed using chlorophyll *a*, determined fluorimetrically after filtration (50 mL) on Millipore filters (0.45 μm) and overnight extraction in 90% acetone at 4°C, according to Stainton et al. (1977). Phytoplankton were preserved in acidic Lugol's iodine solution and analyzed within 7 days of sampling using inverted phase-contrast microscopy by the Utermohl (1958) method. By monitoring the variability of the daily chlorophyll *a* concentrations, I selectively analyzed the Lugol-fixed samples on June 1, 3, 7, 10, and 15 in the enriched enclosures, and on June 1, 3 (chlorophyll maximum), and 15 in the three (Lake, Ctrl, Ctrl < 65 μm) nonenriched treatments. The dominant taxa were identified using Prescott (1961), Huber-Pestalozzi (1941), and Bourrelly (1968). Between 100 and 400 individuals of the dominant taxa were identified and enumerated per sample. Soluble reactive phosphorus (SRP), ammonia-nitrogen, and nitrate and nitrite were determined by the molybdenum blue, the phenol-hypochlorite, and the cadmium-reduction automated methods, respectively (Stainton et al. 1977). Ammonium and SRP were analyzed at 1- to 5-day intervals in the enriched enclosures and 3-day intervals in the nonenriched enclosures. Enclosure sampling ceased after 15 days, when SRP concentrations had stabilized to low values (~0.32 μM) in the ammonia-enriched enclosures. Nitrate and nitrite were determined at 1- to 2-day intervals in the lake and unfiltered control tubes, and on June 1, 3, 5, and 10 in P and PFe treatments. Unfortunately, the nitrate samples for the Ctrl < 65 μm enclosures were lost; however, there is little reason to expect deviations from the nitrate concentrations observed in the unfiltered control tubes. Fe(II) concentrations were determined by the ferrozine method (Gibbs 1979). To minimize stripping of Fe(III), the acidic acetate buffer was replaced by a phosphate buffer (Aubé 1994).

Results

On June 5, 1992, the limnological conditions in lac Tantaré were as follows: temperature, 16.8°C; pH, 5.6; total Fe, 0.69 $\mu mol \cdot L^{-1}$; PAR (400–700 nm) extinction coefficient, $-0.21 \pm 0.01\ m^{-1}$ (mean ± SE); UV (300–400 nm) extinction coefficient, $-0.57 \pm 0.12\ m^{-1}$; total P, 0.43 ± 0.05 $\mu mol \cdot L^{-1}$; total dissolved P, 0.05 ± 0.002 $\mu mol \cdot L^{-1}$.

Phytoplankton excluded from the filtered enclosures (65-μm plankton net) was negligible as revealed by

Table 2. Average ammonium concentrations, initial and final $NO_3 + NO_2$ concentrations, initial PO_4 concentrations and uptake rate constants, and initial Fe(II) concentrations in the experimental treatments.

Treatment	NH_4	$NO_3 + NO_2$ Initial	$NO_3 + NO_2$ Final	PO_4	PO_4 rate constants (day^{-1})	Fe(II)
Lake	0.48±0.14*	3.12	1.6	nd	nd	0.14±0.01
Control	0.28±0.03	3.04	3.03	nd	nd	0.06±0.002
Control < 65μm	0.19±0.08	na	na	nd	nd	0.07±0.002
P	0.14±0.09	3.16	0.36	2.7±0.32	−0.074±0.010	0.11±0.01
PFe	0.12±0.09	3.07	0.01	2.9±0.14	−0.067±0.013	0.23±0.01
NPFe	See text	3.06	3.01	2.0±0.05	−6.75±0.30†	0.23±0.01

Note: nd, not detected; na, not available. Values are given as the mean ± SE, in micromoles per litre.
*$p < 0.05$.
†Linear uptake kinetics.

chlorophyll *a* and initial microscopic analyses carried out on enclosure and intact lake water (Fig. 1). The phytoplanktonic community was dominated by small flagellated chrysophytes, composed mostly of *Chromulina* spp., *Dinobryon* spp. (mostly *D. bavaricum*, but some *D. sertularia*), *Mallomonas* spp. (nearly equal proportions of *M. pr:silio* and *M. caudata* were observed throughout the experiment), and a unicellular coccoid *Synechococcus*-like picocyanobacterium. The cyanobacterium *Merismopedia tenuissima* developed in the ammonium-enriched enclosures toward the end of the experiment.

Chlorophyll *a* concentrations decreased slightly and varied similarly in lake and unfiltered control enclosures, suggesting a negligible enclosure effect on the development of the planktonic community over the course of the experiment (Fig. 1). In contrast, chlorophyll *a* concentrations increased in the herbivore-free control and enriched enclosures relative to the levels in both lake and unfiltered enclosures (Fig. 1). From the initial exponential increases in chlorophyll *a*, phytoplanktonic growth rates were calculated in the herbivore-free and enriched enclosures (Table 1). Enrichment resulted in significantly (Student's *t* test, $p < 0.05$) higher growth rates than in the unenriched controls, but the differences were not significant among the differentially enriched treatments, suggesting that P was responsible for stimulating growth. However, from the doubling in chlorophyll *a* concentrations observed in the herbivore-free control enclosures, it is evident that in early June, the phytoplankton standing crop in the lake was regulated by grazing rather than by a limiting nutrient, in spite of very low total dissolved phosphorus concentrations (<0.05 μM).

In the P- and PFe-enriched enclosures, phytoplanktonic growth continued until June 5 and 7, respectively, at which time nitrates became exhausted and the abundance decreased thereafter. Growth continued until day 10 in the NPFe treatments and then declined.

Ammonium concentrations in the lake were significantly higher (Table 2, one-way analysis of variance (ANOVA)) than in the four other nonenriched treatments, most likely because of continuous diffusion from the sediments, which was absent in the enclosures. The rate of disappearance of ammonium from the two enriched (NPFe) treatments was linear and not significantly different between the duplicate enclosures. Pooled ammonium concentrations yielded the following negative linear relationship with time: NH_4 concentration = -3.86 ± 0.40·days + 65.3 ± 6.3 ($n = 14$, $r^2 = 0.89$).

Natural Fe(II) concentrations measured in the lake samples were unexpectedly nearly twice those measured in the nonenriched enclosures (Table 2) and considerably higher than late-summer samples (~0.02–0.05 μM, J.C. Auclair, unpublished results). More enigmatically, both P-enriched enclosures had higher Fe(II) concentrations than the other nonenriched treatments (see Discussion). Nevertheless, an Fe gradient was clearly established among the experimental treatments as was originally intended.

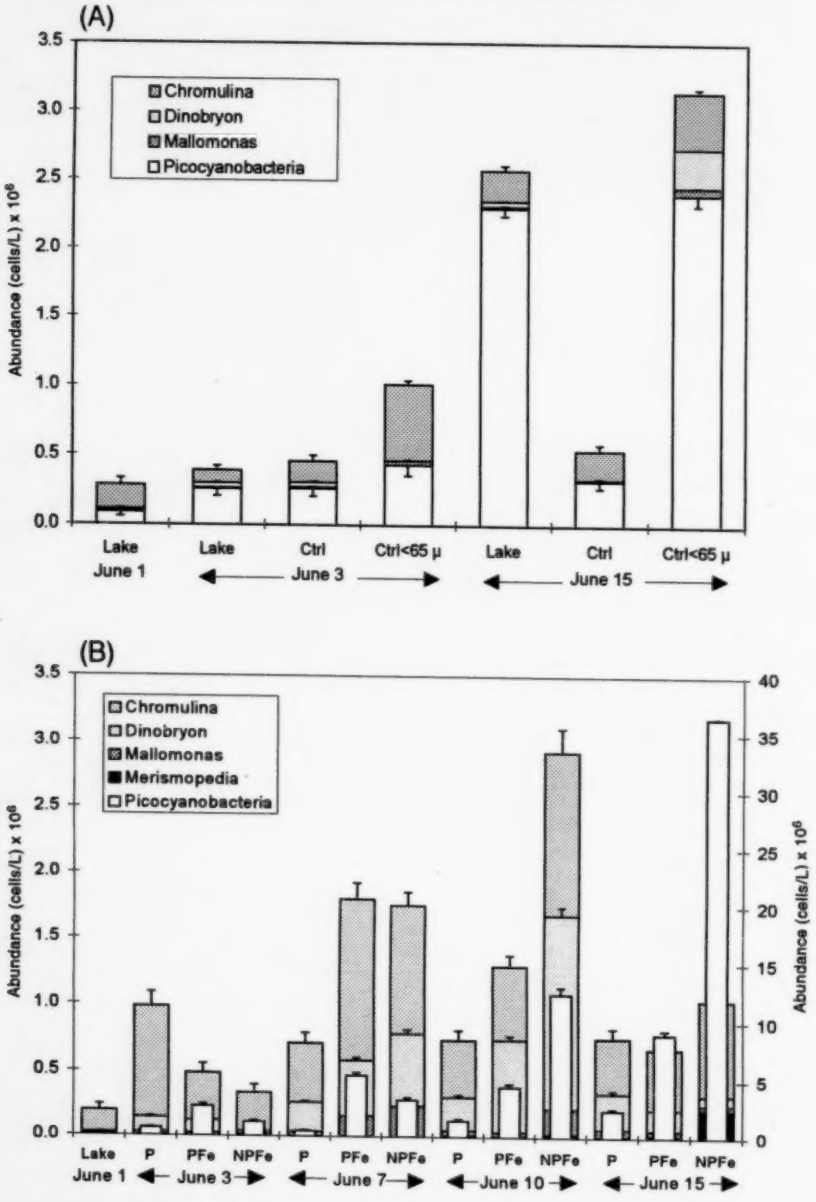
Natural lake nitrate concentrations decreased to trace concentrations by June 10 in both P and PFe treatments, while concentrations remained constant in the control and NPFe enclosures (Table 2). In contrast, a linear (-0.25 μmol·L^{-1}·d^{-1}, $r^2 = 0.99$) disappearance of nitrate from the lake water was observed.

Soluble reactive phosphorus was undetectable (<1 μM) in the nonenriched enclosures. Phosphorus uptake rates were exponential (r^2 range 0.82–0.90) and not significantly different for the P and PFe enrichments, in contrast to the ammonium-enriched enclosures where P uptake was linear and significantly greater than for the other treatments (Table 2). P concentrations were reduced to approximately 0.32 μmol·L^{-1} in these enclosures after 10 days. Thus, at ambient nitrogen concentrations, Fe addition did not significantly alter P uptake rates in the P- and PFe-enriched enclosures.

Phytoplankton species composition and abundance

The proportional abundance of chrysophyte species was similar in the lake and unfiltered control enclosures throughout the experiment. In the herbivore-free nonenriched enclosures, the increase in chlorophyll *a* concentration observed over the first 72 h corresponded with an increased abundance of *Chromulina* spp. and picocyanobacteria (Figs. 1 and 2A). By the end of the experiment, the picocyanobacteria had increased by 5 times in the lake and filtered control enclosures to abundance levels similar to those in the P-enriched enclosures (Fig. 2A). Among the chrysophytes, the relative abundance of *Dinobryon* spp.

Fig. 2. (A) Species composition and abundance in lake (Lake), unfiltered (Ctrl), and filtered (Ctrl < 65 μm) control enclosures on June 1, 3, and 15, 1992. Error bars indicate 95% confidence intervals. (B) Same measurements in the phosphorus (P), phosporus and iron (PFe), and ammonium, phosphorus, and iron (NPFe) enriched enclosures on five dates in June. Note that the right *y*-axis scale applies to picocyanobacteria. Error bars indicate 95% confidence intervals.



and *Mallomonas* spp. became more important at the end of the experiment on June 15. Large species shifts or other enclosure effects between the lake and our control enclosures were minimal over the 15-day study period.

In the enriched enclosures, the total phytoplanktonic abundance as revealed by the chlorophyll *a* concentrations increased progressively in both the PFe and NPFe treatments, relative to the P treatment, throughout the first 10 days of the experiment (Fig. 1B). Picocyanobacterial abundance was not significantly stimulated by P additions relative to the filtered control enclosures on June 3 and 15. However, abundance was always greater in the PFe treatment

than in the P treatment alone, suggesting that Fe additions were responsible for the increased picocyanobacterial growth.

There was a collapse of the chrysophyte population on June 15, concomitant with a 3-fold increase in picocyanobacterial and bacterial abundance (data not shown) and the appearance of the cyanobacterium *Merismopedia tenuissima*.

Discussion

The experiments revealed very rapid phytoplanktonic growth rates, tightly controlled by intense grazing of the crustacean and rotiferan herbivore communities in early June in this oligotrophic lake. For iron to become a significant factor in controlling algal growth in lac Tantaré, the standing crop would need to increase by at least 2–3 times. This is highly unlikely because in situ chlorophyll *a* concentrations are highest in the spring and then decrease throughout the ice-free season. In the absence of grazing my results show that phosphorus controls phytoplankton abundance; however, given the rapid disappearance of nitrate (see below) from the lake water, both nutrients may be growth rate limiting by early July.

Reduced Fe(II) concentrations in the unenriched enclosures relative to the levels in the lake may have been due to low regeneration (absence of sediments) and (or) restricted photoreduction in the enclosures. However, these were open to the atmosphere, and the light transmittance of the tube polyethylene material in both the UV-B (integrated 300–400 nm) and PAR (integrated 400–700 nm) ranges was 80 and 87%, respectively. Turbulence was also limited within the enclosures but it is not known if chromophore photoreduction depends on turbulence. Since a maximum of about 1 nM Fe was contributed by the P stock solutions, the presence of elevated P concentrations must have rendered the autochtonous Fe that was adsorbed or complexed to dissolved humic material more photoreducible.

Nitrate assimilation was low in both the control and ammonium-enriched enclosures. These observations were consistent with the small abundance changes observed in the former, and with uptake inhibition of nitrate in the presence of a more reduced form of nitrogen in the latter (McCarthy 1980). Owing to the shallow depth and high transparency of this lake, a thick hyperbenthic periphytic mat composed of *Mougeotia* spp. develops over the course of the spring as the lake temperature rises. Nutrient exchange measured at the sediment–water interface with benthic chambers (R. Carignan and J.C. Auclair, unpublished results) reveals that nitrate is actively taken up by this community and perhaps by denitrifying bacteria in the sediments. This explains the rapid linear disappearance of nitrate observed from the lake.

Enhanced chrysophyte abundance in response to stimulation of bacterial production in glucose-, nitrogen-, and phosphorus-amended enclosures was also observed in Lake Njupfatet, Sweden, a lake limnologically similar to lac Tantaré (Bell et al. 1993). The enhanced chrysophyte abundance in both PFe and NPFe enclosures observed on June 7 and 10 might thus be due to increased picoplanktonic production in these two treatments. On these two dates, midway through the experiment, the relative proportion of all of the potentially mixotrophic species was remarkably constant in spite of a 10-fold increase in the total abundance of nanoplanktonic organisms over the first 10 days of the experiment. This suggests that intense mixotrophy may have reduced strong competitive interactions among these species. In addition, if the mixotrophic chrysophytes obtain most of their iron from phagotrophy, the rapid growth response of the picoplankton in the presence of elevated iron might buffer the nanoflagellates against iron limitation, resulting in little change in their abundance.

The appearance of *M. tenuissima* is more difficult to explain. Although members of the genus were found in multispecies cyanobacterial mats that displayed nitrogenase activity (Stal et al. 1984), there is no other evidence in the literature that *Merismopedia* spp. fix N_2. Nitrogen limitation should have resulted in an earlier appearance and possibly greater abundance of *M. tenuissima* in the PFe treatment than in the NPFe treatment, while the opposite was observed. Increased heterotrophy from a collapse of the chrysophytes and (or) competitive superiority in the presence of ammonium might also explain the increased success of *M. tenuissima* in the NPFe enclosures. In Lake Njupfatet enclosures, Blomqvist et al. (1989) demonstrated that with ammonium as the nitrogen source, growth of *M. tenuissima* was favored regardless of whether the species was dominant at the onset of the experiments.

In the presence of added P, the addition of Fe(II) quickly stimulated picocyanobacterial production and abundance, while mixotrophic chrysophytes increased more slowly. From the experimental design, it was difficult to discern autotrophic stimulation of chrysophyte production from a stimulation through the microbial food web. However, two observations suggest the latter: (*i*) competitive interactions among mixotrophic species were reduced because their relative abundances were similar midway through the experiment, and (*ii*) a large increase in picocyanobacterial abundance occurred simultaneously with a collapse of the chrysophyte community. Since most organisms constituting the phytoplankton in spring are potentially mixotrophic, their iron quotas may be significantly affected by, if not entirely obtained from, their picoplanktonic prey.

Acknowledgements

Paul Harrison provided technical assistance in the field and laboratory. M. Twiss and A. Tessier commented on and considerably improved earlier drafts of the manuscript, as did an anonymous referee. Finally, I am indebted to the Institut national de la recherche scientifique, for financially supporting this research.

References

Anderson, D.M., and F.M. Morel. 1980. Uptake of Fe(II) by a diatom in oxic culture medium. Mar. Biol. Lett. 1: 263–268.

Aubé, F. 1994. Photoréduction du fer dans la colonne d'eau en milieu lacustre. Thèse de maîtrise en sciences de l'eau, Institut national de la recherche scientifique – eau, Sainte-Foy, Que.

Bell, R.T., K. Vrede, U. Stensdotter-Blomberg, and P. Blomqvist. 1993. Stimulation of the microbial food web in an oligotrophic, slightly acidified lake. Limnol. Oceanogr. 38: 1532–1538.

Blomqvist, P., H. Olsson, H. Olofsson, and O. Broberg. 1989. Enclosure experiments with low-dose additions of phosphorus and nitrogen in the acidified lake Njupfatet, central Sweden. Int. Rev. Gesamten Hydrobiol. 74: 611–631.

Bourrelly, P. 1968. Les algues d'eau douce. Tome II. Les algues jaunes et brunes. Boubee, Paris.

Finden, D.A.S., E. Tipping, G.H.M. Jaworski, and C.S. Reynolds. 1984. Light-induced reduction of natural iron (III) oxide and its relevance to phytoplankton. Nature (London), 309: 783–784.

Francko, D.A., and R.T. Heath. 1982. UV-sensitive complex phosphorus: association with dissolved humic material and iron in a bog lake. Limnol. Oceanogr. 27: 564–569.

Gibbs, M.M. 1979. A simple method for the rapid determination of iron in natural waters. Water Res. 13: 295–297.

Huber-Pestalozzi, G. 1941. Teil 2:1. Chrysophyceen, farblose Flagellaten, Heterokonten. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart. pp. 1–365.

Jackson, T.A., and R.E. Hecky. 1980. Depression of primary productivity by humic matter in lake and reservoir waters of the boreal forest zone. Can. J. Fish. Aquat. Sci. 37: 2300–2317.

Martin, J.H., and S.E. Fitzwater. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature (London), 331: 341–343.

McCarthy, J.J. 1980. Nitrogen. *In* The physiological ecology of phytoplankton. *Edited by* I. Morris. University of California Press, Berkeley, Calif.

Murphy, T.P., D.R.S. Lean, and C. Nalewajko. 1976. Blue-green algae: their excretion of iron-selective chelators enables them to dominate other algae. Science (Washington, D.C.), 192: 900–902.

Murphy, T.P., K.J. Hall, and I. Yesaki. 1983. Biogenic regulation of iron availability in a eutrophic hardwater lake. Sci. Total Environ. 28: 37–50.

Prescott, G.W. 1961. Algae of the western Great lakes area. W.C. Brown Company, Dubuque, Iowa.

Rich, H.W., and F.M.M. Morel. 1990. Availability of well-defined iron colloids to the marine diatom *Thalassiosira weissflogii*. Limnol. Oceanogr. 35: 652–662.

Sakamoto, M. 1971. Chemical factors involved in the control of phytoplankton production in the experimental lakes area, northwestern Ontario. J. Fish. Res. Board Can. 28: 203–213.

Saunders, G.W. 1957. Interrelations of dissolved organic matter and phytoplankton. Bot. Rev. 23: 389–409

Schelske, C.L., F.F. Hooper, and E.J. Haertl. 1962. Responses of a marl lake to chelated iron and fertilizer. Ecology, 43: 646–653.

Schenck, R.C., A. Tessier, and P.G.C. Campbell. 1988. The effect of pH on iron and manganese uptake by a green alga. Limnol. Oceanogr. 33: 538–550.

Shapiro, J. 1957. Chemical and biological studies on the yellow organic acids of lake water. Limnol. Oceanogr. 2: 161–179.

Stainton, M.P., M.J. Capel, and F.A.J. Armstrong. 1977. The chemical analysis of fresh water. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. No. 25.

Stal, L.J., S. Grossberger, and W.E. Krumbein. 1984. Nitrogen fixation associated with the cyanobacterial mat of a marine laminated microbial ecosystem. Mar. Biol. (Berlin), 82: 217–224.

Utermohl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplanktonmethodik. Mitt. Int. Ver. Theor. Angew. Limnol. 9: 1–38.

Wells, M.L., and L.M. Mayer. 1991. The photoconversion of colloidal iron oxyhydroxides in seawater. Deep-Sea Res. 38: 1379–1395.

Wells, M.L., L.M. Mayer, and R.R.L. Guilllard. 1991*a*. A chemical method for estimating the availability of iron to phytoplankton in seawater. Mar. Chem. 33: 23–40.

Wells, M.L., L.M. Mayer, O.F.X. Donard, M.M. de Souza Sierra, and S.G. Ackelson. 1991*b*. The photolysis of colloidal iron in the oceans. Nature (London), 353: 248–250.

Wurtsbaugh, W.A., and A.J. Horne. 1983. Iron in eutrophic Clear Lake, California: its importance for algal nitrogen fixation and growth. Can J. Fish Aquat. Sci. 40: 1419–1429.

Characteristics and function of large woody debris in subalpine Rocky Mountain streams in northern Colorado

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Abstract: Large woody debris has been well studied in coastal forests of the Pacific Northwest, but little is known of its role in Rocky Mountain streams. Large woody debris was measured in 11 undisturbed streams draining subalpine old-growth forests in north central Colorado to assess abundance, characteristics, and function. Although large woody debris in Colorado had smaller diameter, length, and volume than in the Pacific Northwest, its abundance and function were similar. The majority of pools (76%) were plunge and dammed pools formed by large woody debris, most of which spanned the channels perpendicular to stream flow. Smaller streams had a greater proportion of such perpendicular pool-forming pieces than larger streams. Four disturbed streams had significantly less and smaller large woody debris than undisturbed streams. Flows in larger undisturbed streams were capable of moving large woody debris, so pieces were more often located at the stream margins, oriented diagonally, or distributed in clumps than in smaller streams. Individual pools were larger and deeper in larger streams, but their size was not related to the size of large woody debris pieces forming them. Therefore, the function of large woody debris in forming fish habitat in small Rocky Mountain streams is strongly influenced by the stream's location within the watershed.

Résumé : Les gros débris ligneux ont été très étudiés dans les forêts de la côte nord-ouest du Pacifique, mais on sait très peu de choses sur leurs effets dans les cours d'eau des Rocheuses. On a évalué l'abondance, les caractéristiques et la fonction des gros débris ligneux dans 11 cours d'eau non aménagés des vieilles forêts subalpines du centre nord du Colorado. Les débris trouvés dans le Colorado étaient d'un diamètre, d'une longueur et d'un volume moindres que ceux trouvés sur la côte nord-ouest du Pacifique, mais leur abondance et leur fonction étaient comparables. Dans la majorité des cas, les bassins (76%) s'étaient formés au pied d'une chute avec un barrage de gros débris ligneux, la plupart de ces derniers entravant le canal perpendiculairement au courant. Dans les petits cours d'eau, on a trouvé une plus grande proportion de débris perpendiculaires au courant ayant donné lieu à la formation d'un bassin. Les débris ligneux de quatre cours d'eau aménagés étaient significativement moins nombreux et plus petits que ceux des cours d'eau non aménagés. Dans les grands cours d'eau non aménagés, le courant était assez fort pour déplacer les débris, de sorte que, par comparaison aux petits cours d'eau, ceux-ci se trouvaient plus souvent au bord de l'eau, en position diagonale ou accumulés par endroits. Les bassins des grands cours d'eau étaient plus grands et plus profonds, mais leurs dimensions n'étaient pas liées à celles des débris ligneux responsables de leur formation. Par conséquent, la fonction des gros débris ligneux dans la formation de l'habitat des poissons dans les petits cours d'eau des Rocheuses dépend dans une large mesure de l'emplacement de ces cours d'eau dans le bassin versant.
[Traduit par la Rédaction]

Received August 11, 1994. Accepted March 2, 1995.
J12530

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Introduction

Large woody debris (LWD) plays important roles in the structure and function of stream ecosystems. Research conducted primarily in wet coastal forests of the Pacific Northwest indicates that LWD shapes channel morphology (Bisson et al. 1987; Ralph et al. 1994), contributes to the abundance and geometry of pools (Murphy and Hall 1981; Beschta and Platts 1986; Fausch and Northcote

1992), and stores organic and inorganic sediment (Harmon et al. 1986; Bilby and Ward 1989; Nakamura and Swanson 1993). Natural log dams create a stepped channel profile (Bilby and Likens 1980), allowing dissipation of energy at each step and reducing energy available for sediment transport (Heede 1972, 1985). Large woody debris contributes to substrate diversity and creates habitat complexity at various scales (Keller and Swanson 1979), which together are thought to provide critical summer and winter habitat for salmonids (Tschaplinski and Hartman 1983; Johnson et al. 1986; Murphy et al. 1986). Both large and fine woody debris also provide habitat and food for aquatic invertebrates on which fish feed (Angermeier and Karr 1984; Benke et al. 1985).

Although it is evident that LWD plays a complex role in coastal streams of the Pacific Northwest, little information is available on LWD in streams on the east side of coastal mountain ranges and in the Rocky Mountains (cf. Harmon et al. 1986). For example, the characteristics and function of wood in Rocky Mountain streams may differ markedly from the Pacific Northwest because of drier climate and differences in geology, flow regimes, predominant tree species, and tree sizes. As in other regions, many Rocky Mountain streams and associated riparian zones were extensively altered by mining, logging, and railroad tie drives from 1850 to 1940 (Wroten 1956; Young et al. 1994), and human activities continue to affect riparian forests. As a result, relatively few undisturbed lotic systems associated with old-growth forests remain, and little is known about the importance of wood in streams of this region.

The purpose of this study was to describe the characteristics and functions of LWD in undisturbed streams associated with subalpine old-growth forests in the Rocky Mountains of northern Colorado. The climate, flow regime, and tree species of these forests are generally similar to the subalpine Rocky Mountains of the U.S. and Canada, and to the drier east side of the coastal mountain ranges (Alexander 1980), so the results should be broadly applicable to a large region where little is known about the role of LWD. A primary objective of the research was to determine the role of LWD in forming pools and creating overhead cover for salmonids, two elements of habitat that are thought to be important for their survival (White 1975, 1991; Hunt 1993). Understanding how LWD contributes to shaping these habitat features at different spatial scales is critical for managers attempting to restore degraded streams (Reeves et al. 1991).

Study area

Eleven stream reaches associated with old-growth forest were selected for study during the summers of 1992 and 1993 (Table 1). All are located at high elevations (2719–3204 m) in the Arapaho and Roosevelt national forests in north central Colorado (Fig. 1), and flow through glaciated valleys with shallow soils overlying glacial till. These mountains are composed primarily of pre-Cambrian granite, schist, and gneiss (Chronic 1980). Riparian forests were Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*), which are usually found above 2750 m, although this forest type can occur as low as 2440 m in northern Colorado (Hess and Alexander 1986). Most patches of old-growth forest in this region are of this type. Annual precipitation ranges from 64 to 76 cm at 3050 m elevation (Hess and Alexander 1986).

Old-growth forests, which for this region and vegetation type are generally >250 yr old, were identified using six structural characteristics defined by Lowry (1992). Although not all needed to be present, the minimum requirements were (*i*) large live trees (≥35 cm diameter at breast height; dbh), including 15 or more trees ≥30 cm dbh per 0.4 ha; (*ii*) either snags or fallen trees ≥35 cm dbh, with at least two snags or three fallen trees ≥30 cm dbh per 0.4 ha; (*iii*) a multistoried tree canopy; and (*iv*) more than 20% overhead canopy closure.

Stream reaches were selected to meet four additional criteria: (*i*) old-growth spruce–fir forest on both sides of the reach; (*ii*) no major disturbance upstream (i.e., clear-cut logging, fire); (*iii*) no evidence of beaver (*Castor canadensis*) upstream or in the reach; and (*iv*) minimum average bank-full width of 2 m. Reaches that had significant gradient changes or received tributaries were also avoided, because either could affect LWD abundance and characteristics. Reaches selected were generally 250–350 m long and had moderate gradient (<4%) and sinuosity (<1.5; i.e., B channels, as defined by Rosgen 1985). Such channels are common in spruce–fir old growth of the region, and provide a large share of habitat volume for resident trout. Only 13 stream reaches that met these criteria were found in the Arapaho and Roosevelt national forests, of which 11 were studied (Table 1).

Four stream reaches with riparian forests that had been altered by either anthropogenic or natural disturbances were also measured, for comparison to old-growth streams. One is in the Roosevelt National Forest, and three are in the adjacent Routt National Forest to the west (Fig. 1). The disturbances likely occurred before 1900, because the riparian forests had regenerated but were not mature. At two sites, the North Fork Cache la Poudre River and Jack Creek, stumps or historical records indicated that the riparian forest had been cut, but the disturbance could not be determined for the other two sites. The disturbed streams were associated with spruce–fir forests in glacial valleys, and were similar to old-growth reaches in elevation, width, gradient, and sinuosity (Table 1). The four 245- to 250-m reaches were selected in 1987 as control sections for a long-term study of trout population response to habitat enhancement using log drop structures (Riley and Fausch 1995) because they generally lacked LWD.

Methods

Stream channels and salmonid habitat

Reach sinuosity was estimated in the field over three sections (totaling 100–200 m) within study reaches as the thalweg distance divided by the straight-line down-valley distance, measured by tape or range finder. Gradient was determined by using a level and leveling rod in three 50-m sections. Both sinuosity and gradient were measured at the top, middle, and bottom of each reach, and averaged. Habitat units were classified as pool, riffle, or glide, and

Table 1. Characteristics of study reaches and pool habitat in 11 old-growth and 4 disturbed streams in north central Colorado where large woody debris (LWD) was measured.

Stream	Elevation (m)	Stream order	Reach length (m)	Bank-full width (m; mean ± SE)	Gradient (%)	Drainage area (km^2)	Sinuosity[a]	Dominant substrate		Percent pool area	Pools per 10 channel widths	Percent pools formed by LWD	Residual pool depth[b] (cm; mean ± SE)
								2–256 mm	>256 mm				
Old-growth streams													
Tributary of Joe Wright Creek	3204	1	268	3.7±0.14	2.7	3.4	1.04	89	3	12	1.79	62	22±3.14
Tributary of North Fork Joe Wright Creek	3085	1	285	3.8±0.12	6.4	2.4	1.08	73	19	17	2.67	80	28±2.61
North Fork Joe Wright Creek	3036	2	577	3.9±0.07	3.4	3.9	1.20	80	13	18	2.03	90	23±1.85
Hourglass Creek	3109	1	221	4.1±0.14	0.6	5.1	1.14	89	2	16	1.11	67	36±4.89
Fall Creek	2914	3	263	4.1±0.13	3.0	9.9	1.20	61	23	18	1.09	71	28±4.45
Beaver Creek	3011	2	260	4.2±0.11	0.4	8.9	1.34	78	3	30	2.75	88	33±4.10
North Fork Poudre River	2902	1	277	4.5±0.12	2.0	11.0	1.13	49	33	17	1.46	67	26±3.04
Little South Fork Poudre River	2847	3	344	5.6±0.12	2.7	21.7	1.32	80	8	15	1.47	78	39±4.36
Baker Gulch	2743	2	314	7.0±0.26	3.3	17.4	1.04	85	7	22	3.57	81	36±4.50
Bowen Gulch	2829	2	260	8.0±0.21	3.4	24.5	1.13	64	23	20	3.08	30	40±4.28
Arapaho Creek	2719	3	300	10.2±0.51	2.2	29.1	1.20	64	27	11	2.04	83	48±4.28
Disturbed streams													
North Fork Poudre River	2730	2	250	4.0[c]	1.0	28.9	1.22	72	6	7	0.96	17	30±4.92
Colorado Creek	2775	2	245	4.5[c]	2.5	6.9	1.20	61	17	20	2.94	44	25±2.72
Jack Creek	2925	2	245	4.8[c]	0.7	18.2	1.25	94	4	9	1.37	43	22±4.46
Walton Creek	2775	2	245	5.1[c]	1.2	26.1	1.10	63	35	3	1.87	11	25±3.36

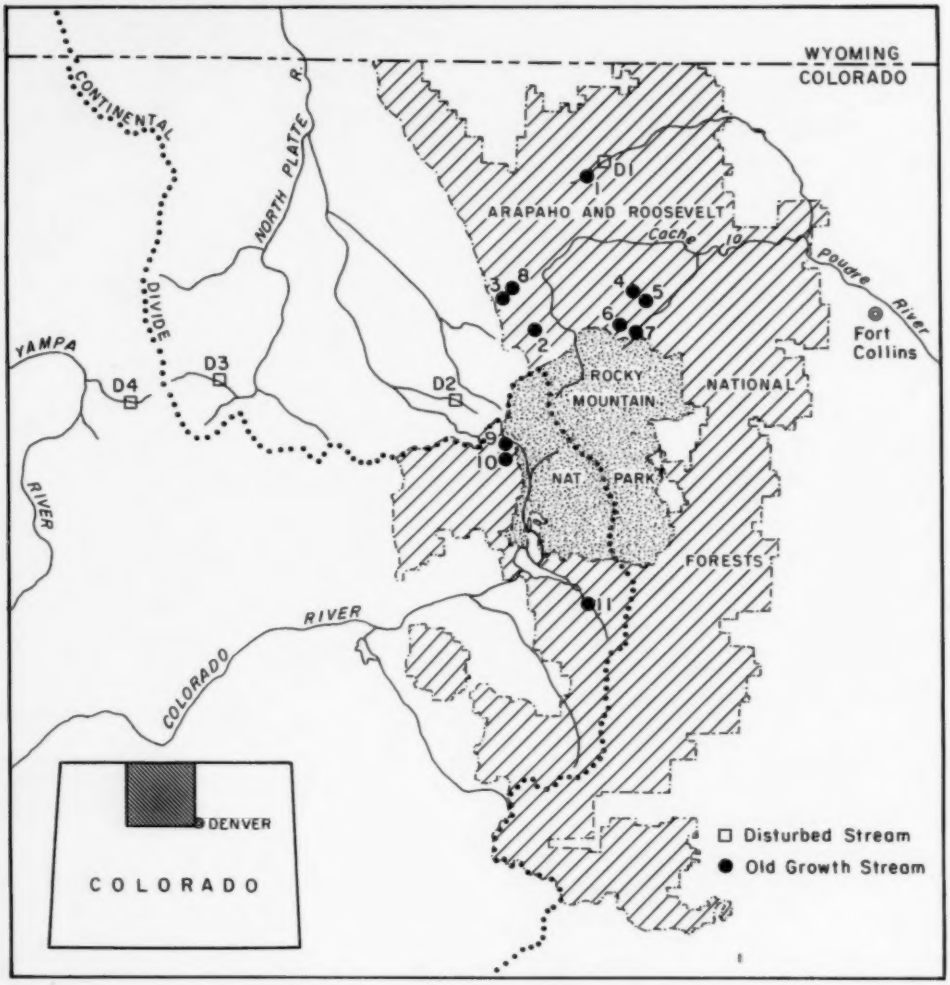
Note: Stream order was determined from U.S. Geological Survey 1: 24 000 topographic maps, based on perennial streams only. The percentage of points along transects where the dominant substrate was visually estimated to be gravel or cobble (2–256 mm diameter) and boulder (>256 mm) is also shown.

[a]Thalweg distance divided by the straight-line down-valley distance.

[b]Maximum depth minus depth of the downstream hydraulic control.

[c]Based on only one transect at each pool (n = 6–16).

Fig. 1. Locations of 11 old-growth and 4 disturbed streams in northern Colorado. Old-growth streams are as follows: 1, North Fork Cache la Poudre River; 2, tributary of Joe Wright Creek; 3, North Fork Joe Wright Creek; 4, Beaver Creek; 5, Hourglass Creek; 6, Fall Creek; 7, Little South Fork Cache la Poudre River; 8, tributary of North Fork Joe Wright Creek; 9, Baker Gulch; 10, Bowen Gulch; 11, Arapaho Creek. Disturbed streams are as follows: D1, North Fork Cache la Poudre River; D2, Jack Creek; D3, Colorado Creek; D4, Walton Creek.



pools were classified as plunge, dammed, lateral scour, or trench pools after Bisson et al. (1982). In addition, pools created by scour of the bed around large woody debris were considered separately.

Wetted and bank-full widths were measured at transects perpendicular to flow at 1-m intervals for pools in all reaches and 2- to 5-m intervals for riffles and glides (longer intervals were used in larger streams). The initial transect in each habitat unit was located half this interval from the downstream end (e.g., 0.5 m from the downstream ends of pools). The diameter of dominant substrates (Orth 1983) was visually estimated at each bank, and one quarter, one half, and three quarters of the stream width along each transect in all habitat types for seven streams measured in 1992, and in riffles and glides for four streams in 1993 (the tributary of North Fork Joe Wright Creek, Baker Gulch, Bowen Gulch, and Arapaho Creek). Substrate was also estimated at seven equidistant points along each transect in pools for the four streams in 1993.

Residual pool depth (Lisle 1987), an index of pool volume that is relatively independent of discharge, was estimated as maximum depth minus depth of the downstream hydraulic control. Length of overhead log or bank cover that could conceal a salmonid 15 cm long from overhead view was also measured, on the basis of criteria in Fausch and Northcote (1992). Percent habitat area, wetted

width, overhead log and bank cover, and substrate percentages for the four disturbed stream reaches were obtained from Riley and Fausch (1995, and unpublished results). Sinuosity, gradient, pool types, pool bank-full width, and residual pool depths were measured for these streams during August 1993.

Large woody debris

LWD was defined as logs ≥10 cm diameter and ≥1 m long. Although larger minimum diameters and lengths have been used by other researchers (e.g., Robison and Beschta 1990*a*, 1990*b*; Young et al. 1990), there are no standard criteria. We used the minimum sizes given in the literature (e.g., Andrus et al. 1988; Fausch and Northcote 1992) because small pieces may play important roles in small mountain streams, and LWD is likely to be smaller in Rocky Mountain than Pacific Northwest streams because of differences in tree species and growing conditions. Pieces that met these criteria were measured if any part occurred within or was suspended above the bank-full stream channel. The diameter at each end and length with diameter ≥10 cm, excluding root wads, was measured using a tree caliper and tape measure. Piece volume was estimated from the equation in Lienkaemper and Swanson (1987):

$$\text{Volume} = \frac{\pi\left(D_1^2 + D_2^2\right)L}{8}$$

where D_1 and D_2 are end diameters (m) and L is length (m).

The relation of each LWD piece to the channel was divided into four influence zones, modified from Robison and Beschta (1990*a*; see also Ralph et al. 1994): (A) completely within the wetted channel, (B) partially within the wetted channel, (C) wholly between the wetted and bank-full channel margins, and (D) wholly above the bank-full channel. Woody debris pieces wholly above the bank-full channel had no effect on channel morphology below bank-full discharge, but may affect it at flood flows and will eventually enter the channel when decay causes breakage. All streams were measured during low flow in mid to late summer.

The orientation and position of LWD pieces within the channel were also recorded. Orientation affects stream flow characteristics and channel morphology, and both characteristics affect piece stability (Hogan 1984; Robison and Beschta 1990*a*). Piece orientation was divided into four classes on the basis of the angle relative to the direction of stream flow: 0° (parallel to flow), 90° (perpendicular), and 45 and 135° (the two diagonal orientations). Pieces were recorded in the class that most closely reflected their true orientation. Four categories were used to describe LWD position: touching the left or right banks only, touching neither bank (midchannel), or touching both banks (spanning).

The apparent function of each LWD piece was classified as forming pools, storing sediment, providing overhead cover for salmonids, having other functions (i.e., bank stability, catching other organic debris), or having no apparent function. Because LWD may serve one or more function, only the last category was mutually exclusive of the rest. The relative age of LWD pieces was assessed using decay classes modified from Grette (1985; see Young et al. 1994) on the basis of the change in piece form and condition. Age was assumed to increase as pieces first lost bark, then branches, and then became slightly or extensively rotted or broke apart as decay progressed.

Statistical analysis

Relationships among LWD and stream channels were analyzed at three scales, individual LWD pieces, pools, and stream reaches, and the results are presented in this order below. Nonparametric statistical analyses were used for LWD diameter, length, and volume because distributions were skewed. For stream reaches, associations between salmonid habitat characteristics (e.g., percentage pool area or length of overhead log cover) and three types of variables (stream size, gradient, and LWD abundance, total volume, or bank-full volume) were analyzed using simple and multiple linear correlation. Multiple correlation coefficients (R) reported here are analogous to simple correlation coefficients (r) in that they measure the strength of association between one variable and several others, but differ in that they range only from 0 to 1 (signs of partial correlation coefficients are reported as needed). Akaike's information criterion (Akaike 1985; Burnham and Anderson 1992) and Mallow's Cp statistic were used to select the most parsimonious sets of variables for multiple correlation, and residual plots were inspected for normality, homogeneous variance, and outliers. Variables that were percentages were subjected to a logit transformation ($\ln(p/1\text{-}p)$, where p is the proportion) to stabilize variance and improve normality if outside the range 20–80%. All except the percentage of LWD pieces in various influence zones were so transformed.

The effects of stream width, gradient, and characteristics of LWD (diameter, length, bank-full volume) on the area and residual depth of 72 individual pools created by single LWD pieces were analyzed using a split-plot analysis of variance (ANOVA, Gill 1978) via the general linear models algorithm in SAS (SAS Institute Inc. 1992). This analysis was needed because pools were, of necessity, not randomly chosen, but instead represented incomplete blocks of relatively homogeneous units within streams. The split-plot ANOVA allowed accounting for stream-to-stream variability in width and gradient (i.e., whole-plot effects) before considering the effect of LWD diameter, length, or volume on individual pool area or depth within streams (subplot effects).

The distribution of LWD pieces along the 11 old-growth reaches was compared with a random distribution using randomization tests (Manly 1991). Monte Carlo simulation was used to assign the number of pieces found in a reach to 3-m intervals at random and repeated 1000 times. The maximum number of pieces actually found in any 3-m interval, and the number of intervals that held at least four times the expected number of pieces based on a uniform distribution, were compared with the distributions of these test statistics generated by the simulations to judge significance.

Results

Characteristics of study reaches

All study reaches were 1st- to 3rd-order streams (Strahler 1957) with mean bank-full widths of 3.7 to 10.2 m, moderate gradient (0.4–6%), and low sinuosity (<1.35; Table 1).

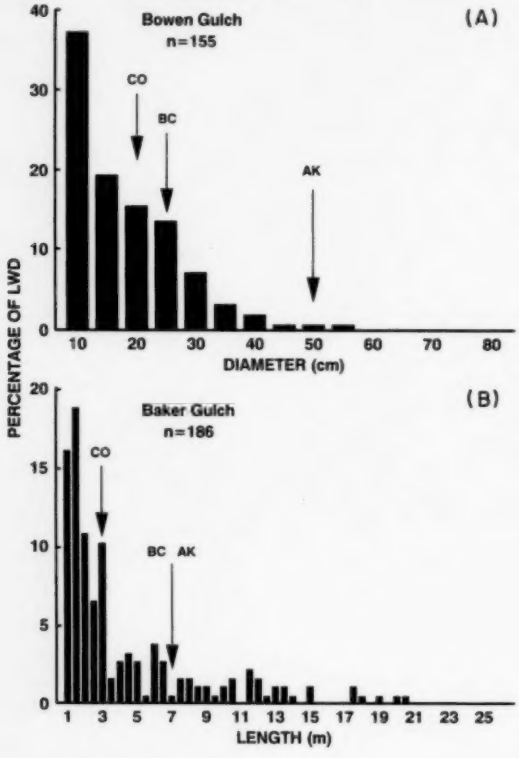


Fig. 2. Distribution of diameter (A) and length (B) of large woody debris pieces (e.g., 10–14.9 cm or 1–1.4 m, etc.) in Bowen and Baker gulches, which are representative of the 11 old-growth streams. The diameter and length of large woody debris pieces in the 11 Colorado streams (CO, medians), in a relatively undisturbed stream section in British Columbia (BC, geometric means), and in 5 undisturbed streams in Alaska (AK, means) are shown for comparison (see text).

Reach lengths ranged from 221 to 575 m, but were 260 to 345 m for 9 of 11 reaches. Pools made up 11–22% of wetted area in all but one low-gradient (0.4%) stream, Beaver Creek (30% pool area). Gravel and cobble (2–256 mm diameter) were the most prevalent substrates in all streams (i.e., visually estimated as dominant substrate at 49–89% of points measured, n = 403–1312; Table 1), although in four streams with >2% gradient, boulder was also prevalent (23–33% of points).

Large woody debris pieces

Distributions of LWD diameters and lengths in the old-growth reaches showed the highest percentages in the smallest size-classes (Fig. 2), so medians were used for comparison among streams. Median LWD diameters were similar for all reaches, ranging from 16 to 22 cm (Table 2). Few pieces in any stream were larger than 50 cm. Median piece length for 10 of 11 reaches ranged from 2.1 to 3.7 m but was 7.7 m for the tributary of Joe Wright Creek. Only about 1% of the 1412 pieces measured were longer than 20 m. Median LWD volume reflected the variability in piece length, ranging from 0.058 m^3 for Bowen Gulch to 0.407 m^3 for the tributary of Joe Wright Creek.

LWD total volume, bank-full volume, and abundance also varied among old-growth reaches (Table 2). Total LWD volume per 100 m of stream averaged 13.3 m^3 (range 6.6–27.1 m^3). These estimates included portions of LWD pieces extending beyond the bank-full channel and thus not currently affecting fish habitat or channel morphology except at the highest flows. This terrestrial portion ranged from 23 to 64% of LWD volume. Total volume of LWD within the bank-full channel averaged 139.5 $m^3 \cdot ha^{-1}$, ranging from 91.5 to 186.5 $m^3 \cdot ha^{-1}$, except for the tributary of the North Fork Joe Wright Creek, which had almost 100% more than the average (254.3 $m^3 \cdot ha^{-1}$). LWD abundance averaged 43 pieces per 100 m of stream but ranged widely, from 18 to 64 pieces per 100 m. Much of the LWD was apparently old, because the percentage of pieces classified as extensively rotted or breaking apart ranged from 56 to 78%.

LWD in the four disturbed stream reaches was less abundant and smaller than in old-growth streams (Table 2), except in Walton Creek, which had large pieces but only five in 245 m. Abundance ranged from 2 to 26 pieces per 100 m, which was significantly less than in old-growth reaches (P = 0.02 by Wilcoxon rank sums test). Length and volume of LWD pieces were also significantly smaller in disturbed streams (P < 0.04 for both), and the smaller average LWD diameter was nearly significant (P = 0.058). Total woody debris volume per 100 m was also significantly less in disturbed than in old-growth streams (P = 0.006).

Pools

Large woody debris that formed pools

LWD pieces that were classified as forming pools in old-growth reaches were larger than debris pieces that were not. The median diameter of pool-forming pieces was 22 cm (on the basis of average piece diameter), compared with 19 cm for all other pieces. The median length of pool-forming pieces was 4.4 m compared with 2.6 m for all other pieces, and median piece volume was 0.186 m^3 for pool-forming pieces versus 0.079 m^3 for all other pieces. All of these differences were significant (P = 0.0001 by Wilcoxon rank sums test).

Position within the bank-full channel and orientation in relation to stream flow also differed between pool-forming and all other LWD pieces. The majority of pool-forming pieces (53%) spanned the channel, touching both left and right channel banks, whereas only 12% of all other woody debris pieces were spanning (Fig. 3). Similarly, 57% of pool-forming debris pieces were oriented perpendicular to stream flow compared with 23% of all other wood pieces. These distributions of pool-forming pieces among position and orientation classes were significantly different from distributions for all other pieces (P < 0.001 for both by G test). The distribution of pool-forming debris pieces among the five decay classes was not significantly different from that of all other pieces (P = 0.36 by G test).

Agents forming pools

A total of 144 pools were measured in the 11 old-growth reaches, of which 76% (n = 110) were formed by LWD

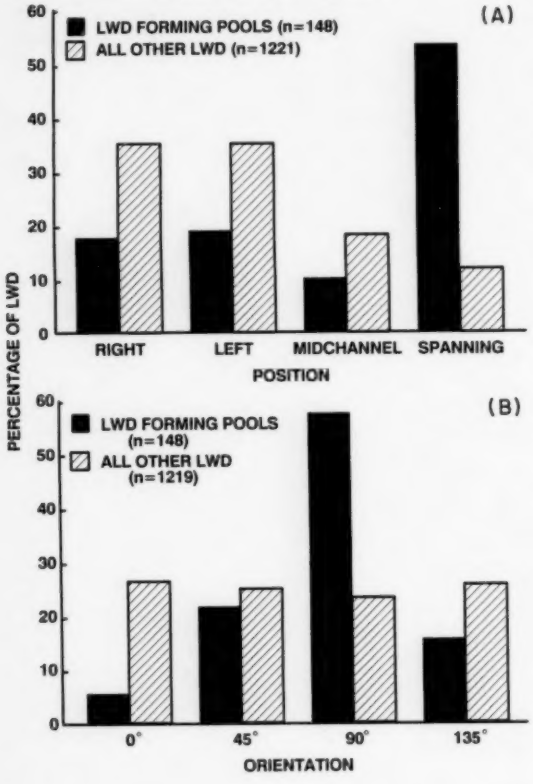
Table 2. Characteristics of large woody debris (LWD) measured in study reaches of 11 old-growth and 4 disturbed streams in north central Colorado.

Stream	Median			Volume		Pieces per 100 m	Percent decay		Percent spanning channel	Percent LWD by orientation		Percent LWD by function		LWD clumps			
														4 × average		Maximum	
	Length (m)	Diameter (m)	Volume (m^3)	Total ($m^3 \cdot 100\ m^{-1}$)	Bankfull ($m^3 \cdot ha^{-1}$)		B	ER+O		Perp.	Diag.	Pool	Cover	*P*	No.	*P*	No.
Old-growth streams																	
Tributary of Joe Wright Creek	7.7	0.22	0.407	9.3	91.5	18	13	67	60	51	20	15	15	0.660	3	0.001	7
Tributary of North Fork Joe Wright Creek	3.1	0.21	0.119	20.7	254.3	50	<1	63	24	29	46	12	6	1.000	7	0.970	6
North Fork Joe Wright Creek	3.1	0.20	0.115	6.6	93.3	24	6	77	22	38	41	20	8	<0.002	4	0.040	7
Hourglass Creek	2.9	0.22	0.107	12.8	134.8	33	10	68	6	18	59	10	18	0.320	5	0.060	7
Fall Creek	3.7	0.19	0.108	14.3	186.5	46	16	59	26	34	49	6	15	0.350	6	0.300	7
Beaver Creek	2.3	0.18	0.069	9.1	165.8	54	6	70	19	33	41	7	35	0.010	7	<0.002	15
North Fork Poudre River	2.8	0.20	0.239	12.6	123.0	37	11	72	25	32	55	16	22	0.030	5	0.040	8
Little South Fork Poudre River	3.3	0.18	0.075	11.8	112.8	32	18	59	11	24	66	6	14	0.002	5	<0.005	14
Baker Gulch	2.8	0.16	0.062	11.7	106.3	59	<1	75	14	27	49	9	6	0.020	8	<0.006	15
Bowen Gulch	2.1	0.18	0.058	10.5	94.5	60	<1	3	3	17	53	4	8	0.010	8	<0.002	14
Arapaho Creek[a]	2.6	0.20	0.085	27.1	171.3	64	1	5	5	17	51	4	10	<0.004	9	<0.001	14
Disturbed streams																	
North Fork Poudre River	2.7	0.16	0.057	3.1	—	22	2	13	13	27	40	5	23	—	—	—	—
Colorado Creek	2.8	0.20	0.095	6.6	—	26	0	21	21	34	43	13	9	—	—	—	—
Jack Creek	1.7	0.15	0.036	1.3	—	16	5	3	3	46	33	10	14	—	—	—	—
Walton Creek	5.8	0.24	0.223	0.6	—	2	0	20	20	60	40	17	17	—	—	—	—

Note: Streams are arranged in order by width, as in Table 1. The probability (*P*) of finding a given number of clumps (No.) that had at least four times the average number of pieces expected, and the probability of finding a clump as large as the maximum number of pieces found, are shown (see text). The percentage of LWD pieces in two of five decay classes (B, bark intact; ER+O, extensively rotted and broken classes combined), and two of four orientation classes (Perp., perpendicular; Diag., diagonal) is also shown. Bank-full volume of LWD was not measured and LWD clumping was not analyzed in disturbed streams.

[a]Excluding LWD pieces in two large accumulations (see text).

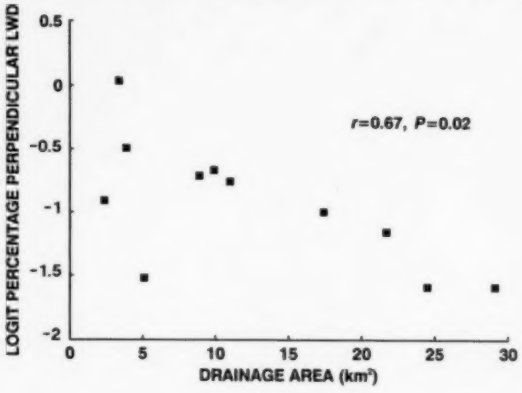
Fig. 3. Distribution of pool-forming large woody debris and all other large woody debris pieces by position (A) and orientation (B) for 11 old-growth streams. Position classes are as follows: touching right bank only, touching left bank only, touching neither bank (midchannel), and touching both banks (spanning). Orientation classes in degrees are as follows: 0° is parallel to stream flow, 90° is perpendicular to stream flow, and 45 and 135° are the two classes diagonal to flow (see text).



(Table 1). Of these LWD pools, the most prevalent were plunge pools (57%) and dammed pools (23%), with fewer scour pools (16%) and trench pools (3%). Of the 34 pools not formed by LWD, 35% were plunge pools formed by boulders and small woody debris (<10 cm diameter), lateral scour and trench pools each made up 29%, and 3% were scour pools. Pool abundance, standardized per 10 channel widths, ranged from 1.09 for Fall Creek to 3.57 for Baker Gulch (Table 1).

In contrast to the old-growth streams, only 32% of the 38 pools in the four disturbed streams were formed by LWD. The distribution of pool types found in the disturbed streams differed significantly from that in the old-growth streams ($P = 0.02$ by G test). Seventy-five percent of the 12 pools formed by woody debris were plunge pools, 17% were dammed pools, and 8% were scour pools. Of the 26 pools formed by other agents (i.e., boulders, meander bends), 38% were trench pools, 23% were scour pools, 19% were lateral scour pools, and 20% were dammed or

Fig. 4. Percentage of large woody debris pieces lying perpendicular to stream flow as a function of drainage area for 11 old-growth streams (see text for logit transformation). Untransformed percentages of perpendicular large woody debris ranged from 17 to 51%.



plunge pools. Pool abundance per 10 channel widths in disturbed streams varied widely, but was not significantly different from that in old-growth streams ($P = 0.48$ by Wilcoxon rank sums test; Table 1). However, the percentage of area as pools in three of four disturbed streams (3–9%) was substantially less than in old-growth streams (median 17%, range 11–30%). The fourth stream, Colorado Creek, had 20% pool area, but also had more LWD than the other disturbed streams (Table 2). Residual pool depth in the disturbed streams was not significantly different ($P = 0.40$ by Wilcoxon rank sums test) from that in the seven old-growth streams of similar bank-full widths (3.7–5.6 m) and gradients (0.4–3.4%).

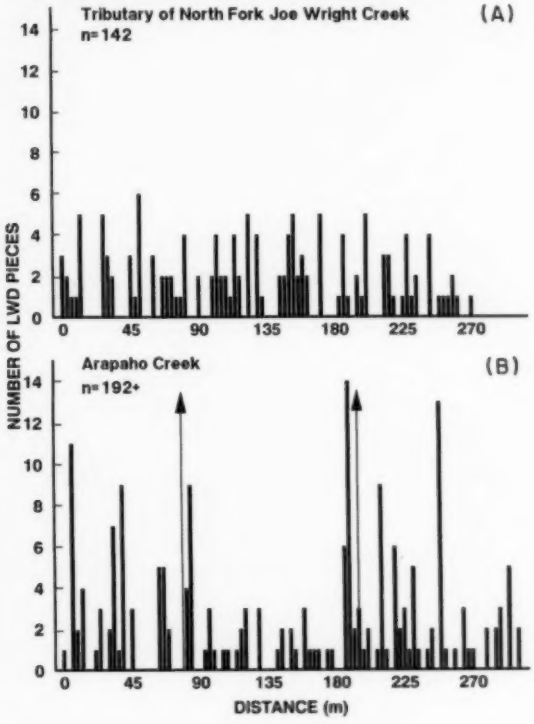
Stream reaches

Characteristics of large woody debris

Characteristics of LWD pieces were related to larger-scale stream characteristics, such as gradient and stream size. The number of LWD pieces per 100 m was greater in larger than smaller streams ($r = 0.62$, $P = 0.04$ for drainage area; $r = 0.69$, $P = 0.02$ for bank-full width). The percentage of pieces spanning the channel decreased in larger streams with larger drainage area or bank-full width (Table 2; $r = -0.70$, $P = 0.02$ for both after logit transformation). Relatively little wood was midchannel where it touched neither bank, except in the Little South Fork Cache la Poudre River (hereafter Little South Fork Poudre) and Beaver and Arapaho creeks, which had debris jams. The percentage of pieces lying perpendicular to the channel also decreased with drainage area (Fig. 4; $r = -0.67$, $P = 0.02$) and bank-full width ($r = -0.69$, $P = 0.02$).

The location of LWD within the bank-full channel and the distribution of debris clumps along reaches were also related to stream size. The percentage of debris pieces at least partially within the wetted channel decreased in larger streams ($r = -0.79$, $P = 0.004$ for drainage area; $r = -0.59$, $P = 0.05$ for bank-full width). In contrast, the percentage of LWD pieces above the wetted channel but still within the

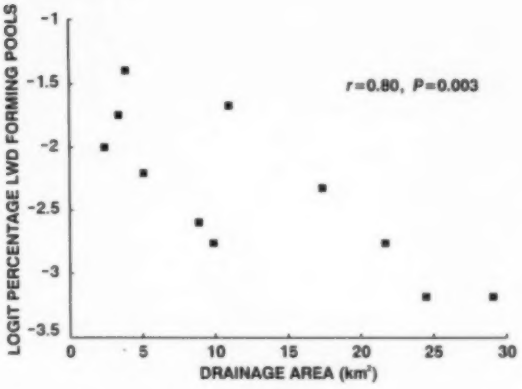
Fig. 5. Frequency distribution of large woody debris pieces in 3-m intervals along study reaches in the tributary of North Fork Joe Wright Creek (A), representative of seven streams of <5.0 m bank-full width, and Arapaho Creek (B), representative of four larger streams. Arrows indicate locations of two very large accumulations in Arapaho Creek (see text).



bank-full channel increased with drainage area ($r = 0.78$, $P = 0.004$) and bank-full width ($r = 0.82$, $P = 0.002$), indicating that in larger streams less LWD influenced fish habitat at low flow. Less wood was suspended above the bank-full channel in the three streams ≥7.0 m bank-full width (3–7%; Baker Gulch, Bowen Gulch, and Arapaho Creek) than in six of the eight smaller streams (10–34%), although North Fork Joe Wright Creek and its tributary had only 4–5% of debris pieces in this category. However, these differences were not significant for either drainage area or bank-full width ($P > 0.27$).

In the seven streams <5.0 m bank-full width, LWD was often distributed relatively evenly along the reach, whereas in the four larger streams the distribution of debris was usually clumped (Fig. 5). In three of seven small streams the maximum frequency of LWD pieces in 3-m intervals was no different from that expected by chance ($P > 0.05$ by randomization test based on 1000 simulations), and the frequency of clumps that were at least four times the average number of LWD pieces was no different from that expected by chance in four of seven streams (Table 2). In contrast, the maximum frequency of LWD pieces was much different from that expected by chance in all four larger

Fig. 6. Percentage of large woody debris pieces forming pools as a function of drainage area for 11 old-growth streams (see text for logit transformation). Untransformed percentages of large woody debris forming pools ranged from 4 to 20%.



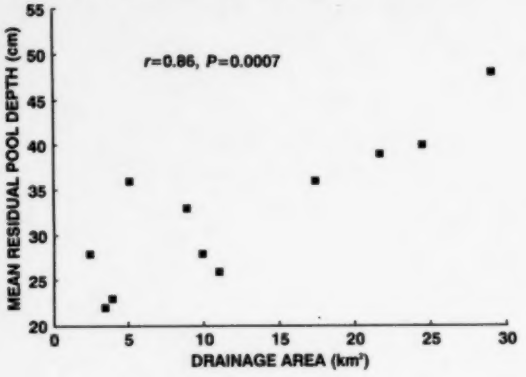
streams ($P < 0.001$ for all), as was the frequency of clumps that were at least four times the average ($P \leq 0.02$ for all). Arapaho Creek, the largest stream measured, also had two very large debris accumulations within the reach that were not included in this analysis (Fig. 5). They were so large that it was impossible to count individual pieces because most were buried. These debris jams measured 16 m wide, 11.5 m long, and 2.5 m high (460 m^3) and 32 m wide, 14 m long, and 1.5 m high (672 m^3) and were spaced approximately 120 m apart. There were five more large accumulations of LWD and four smaller ones in a 3.2-km section bordered by old growth that extended both upstream and downstream of the reach.

Function of large woody debris pieces

The percentage of pieces functioning to form pools and create overhead cover for fish was also related to stream size or gradient. Although only an average of 10% (range 4–20%) of the 1412 LWD pieces functioned to form pools, this percentage was significantly negatively correlated with drainage area (Fig. 6; $r = -0.80$, $P = 0.003$) and bank-full width ($r = -0.71$, $P = 0.01$), indicating that more of the LWD in smaller streams formed pools (Table 2). Similarly, although only 14% of LWD pieces functioned as cover (range: 6–35%), multiple correlation analysis indicated that smaller streams, and those with lower gradients, had a higher percentage of pieces functioning as overhead cover. The interaction term, bank-full width times gradient, was the single variable most closely associated with the percentage of pieces forming cover ($r = -0.86$, $P = 0.0006$), although a multiple correlation with both bank-full width and gradient was also significant ($R = 0.88$, $P = 0.002$; both partial correlations were negative).

LWD provided more overhead cover for trout in low-gradient streams that had more LWD. Three significant multiple correlations were found between length of log cover and two or three variables including stream width or area, gradient, LWD abundance or bank-full volume,

Fig. 7. Mean residual pool depth as a function of drainage area for 11 old-growth streams.



and products of two of these variables (R = 0.77–0.92, P = 0.003–0.025; see Richmond 1994 for details) but in all cases partial correlation coefficients were negative for gradient and positive for LWD abundance or volume. Overhead cover provided by undercut stream banks was also more plentiful in low-gradient streams with more LWD. As for log cover, partial correlation coefficients with gradient were always negative and with LWD abundance were always positive for three significant associations between length of bank cover and one to three of the variables described above (r or R = 0.79–0.92, P = 0.001–0.004).

Characteristics of pools and channels

The percentage of stream area as pools (Table 1) increased with decreasing stream size and increasing LWD abundance. Percentage pool area was positively correlated with LWD abundance per 100 m and negatively correlated with the interaction term, LWD abundance times bank-full width (R = 0.75, P = 0.035). Percentage pool area was not significantly associated with LWD volume alone, or in combination with gradient and width or drainage area (P > 0.05).

Several other characteristics of pools were also related to LWD, stream size, and gradient. Streams with more LWD per 100 m had more pools per 10 channel widths (r = 0.60, P = 0.05), although the relationship was imprecise. However, pool frequency was not significantly associated with total or bank-full LWD volume, alone or with other variables (P > 0.05). Residual pool depth, which averaged 33 cm (Table 1), was positively correlated with bank-full width or drainage area (r = 0.86, P = 0.0007 for both; Fig. 7), as was pool surface area (r = 0.57–0.59, P < 0.00001 for both), although the latter relationships were less precise. Higher gradient streams had more plunge pools (r = 0.60, P = 0.05). Percentage of plunge pools ranged from 17% for Hourglass (0.6% gradient) and Arapaho (2.2%) creeks to 75% for the tributary of North Fork Joe Wright Creek (6.4%) and Baker Gulch (3.3%).

After accounting for the effects of width and gradient among streams, neither pool surface area nor residual depth of 72 pools was significantly related (P > 0.13) to the diameter, length, or bank-full volume of single LWD pieces functioning to form the pools, on the basis of a split-plot ANOVA. In a separate analysis, the mean diameter of single pool-forming pieces was not significantly different among streams (P = 0.45 by one-way ANOVA), but these pieces were significantly longer in Arapaho Creek, the largest stream, than in 5 of 10 smaller streams (P = 0.0005 by ANOVA overall; P < 0.05 by Tukey's honestly significant difference multiple comparisons). The bank-full volume of pool-forming pieces was different among streams (P = 0.0001) but only in Arapaho Creek, the largest stream, were pool-forming pieces of significantly greater volume than in all other streams except Fall Creek (P < 0.05 by multiple comparisons).

Of the 110 pools formed by LWD in the 11 old-growth streams, 105 could be attributed to specific pieces. Seventy-four pools (70%) were formed by single debris pieces, 21 (20%) were formed by two pieces, and 10 (10%) were formed by three or more pieces. Data on the specific pieces were not recorded for the remaining five pools. A significantly higher percentage of pools were formed by single versus multiple LWD pieces in the seven streams of <5.0 m bank-full width than in the four larger streams (P = 0.026 by one-way ANOVA). Seventy-five percent of LWD pools in smaller streams were formed by single pieces, compared with 47% in larger streams.

Discussion

Large woody debris pieces

Direct comparisons of LWD characteristics among studies are complicated by different criteria for minimum size among researchers. Although a 10 cm diameter and 1 m length have often been used (Long 1987; Andrus et al. 1988; Fausch and Northcote 1992), minimum criteria commonly ranged from 10 to 20 cm diameter and from 1 to 3 m length. However, tree species in Colorado are not as large as those in the Pacific Northwest, so excluding LWD pieces <20 cm in diameter eliminated more than half those in old-growth streams (decreased from 43 to 18 pieces per 100 m, on average). Small pieces play important roles in small Colorado mountain streams, so we judged it inappropriate to exclude them from analysis, but differences in criteria must be considered when comparing studies.

Comparisons are also complicated by the use of different descriptive statistics (i.e., mean, geometric mean, median). For example, given the skewed distributions for diameter and length of LWD (Fig. 2; see also Fausch and Northcote 1992), arithmetic means will usually be larger than medians or geometric means. However, in this study, the maximum difference among these three statistics from the 11 old-growth streams averaged only 2 cm (range 1–3 cm) for LWD diameter, and only 1.7 m (range 1.0–2.7 m) for LWD length.

Despite differences in criteria and statistics, LWD in 11 stream reaches draining old-growth subalpine forest in the Rocky Mountains of Colorado was smaller than that in the wet coastal forests of the Pacific Northwest (cf. Harmon et al. 1986), owing to differences in predominant tree species and climate between the two regions. On average, median LWD diameter was 19 cm and median length 3.3 m in Colorado, compared with 53 cm average

diameter and 7.4 m average length in five undisturbed streams in southeast Alaska (Fig. 2; Robison and Beschta 1990*a*). Similarly, LWD in relatively undisturbed reaches of a British Columbia stream had a geometric mean diameter of 26 cm and geometric mean length of 7.4 m (Fausch and Northcote 1992). Median LWD piece volume averaged 0.131 m^3 in the Colorado streams compared with an average of 1.7 m^3 in the five Alaska streams (Robison and Beschta 1990*a*), nearly an order of magnitude difference despite the different statistics used. LWD volume per 100 m of stream averaged 58 m^3 in the five Alaska streams and 43.2 m^3 in the British Columbia stream, compared with 13.3 m^3 in the 11 reaches in Colorado. Similarly, mean bank-full volume was more than four times greater in the British Columbia stream than the Colorado reaches (586.3 vs. 139.5 $m^3 \cdot ha^{-1}$). In contrast to these differences, LWD in the 11 Colorado streams had a similar size distribution to trees in undisturbed Engelmann spruce–subalpine fir riparian forests in southeastern Wyoming (Young et al. 1990), where few trees exceeded 50 cm diameter.

Although LWD was smaller in Colorado streams, average abundance (43 pieces per 100 m) was comparable to values in Alaska (33 pieces per 100 m) and British Columbia (42 pieces per 100 m). Murphy and Koski (1989) also found mean LWD abundances of 30–33 pieces per 100 m in streams of similar channel type to ones we measured, for seven old-growth watersheds in southeast Alaska. Robison and Beschta (1990*a*) set larger minimum size criteria for LWD (>20 cm diameter, >1.5 m length) than we did, as did Murphy and Koski (1989; >10 cm diameter, ≥3 m length), which could account for the lower abundance in the Alaska streams.

LWD in the four disturbed streams was less abundant and smaller than in old-growth reaches. Young et al. (1994) also reported lower debris abundance in three streams where logs for railroad ties had been cut and driven downstream than in paired undisturbed streams of southeastern Wyoming. Debris was significantly larger and more abundant in old-growth than second-growth streams 7–10 m wide in southwestern Washington, but no differences were found for smaller streams (Bilby and Ward 1991). Ralph et al. (1994) found no difference in LWD abundance between logged and unlogged streams, but did find more large LWD pieces (>50 cm) in unlogged basins, where the percentage of large pieces increased with basin area. In each of these cases, direct removal of LWD from channels (i.e., stream cleaning; see Fausch and Northcote 1992) may have played as large a role as reduced input of LWD from the riparian forest owing to logging or fire.

Pools

LWD must attain stability in streams to affect channel morphology and shape fish habitat. Longer debris pieces (relative to stream channel width) are more likely to achieve stable positions because they span the channel and become anchored on stream banks, whereas smaller unstable pieces move during the high flows that shape stream channels (Bryant 1983). In this study, LWD that formed pools was significantly longer and of larger diameter than all other pieces, and the majority spanned the channel perpendicular to flow. Stable perpendicular pieces within the bank-full channel offer greater resistance to flow than pieces in other orientations and positions and are therefore more likely to cause the localized erosion and deposition that forms pools (Sullivan et al. 1987).

Although only about 10% of the 1412 LWD pieces measured functioned to form pools, most of the pools in the study reaches were formed by LWD. Young et al. (1990) also found that LWD formed 70% of pools in an undisturbed stream in southeastern Wyoming, and other researchers have found LWD to be of primary importance in pool formation in other regions (Heifetz et al. 1986; Murphy et al. 1986; Andrus et al. 1988; Fausch and Northcote 1992; Ralph et al. 1994). Like Murphy and Koski (1989) we found that most LWD pieces were decayed and apparently quite old. However, there was no difference in the relative decay of pool-forming LWD and all other pieces, indicating that even the youngest debris pieces that attain stability can form pools.

Stream reaches

Characteristics and function of large woody debris across streams

Among the Colorado streams, larger streams had more LWD per 100 m than smaller streams. Robison and Beschta (1990*b*) and Murphy and Koski (1989) also found greater LWD abundance in larger streams in Alaska, although their streams were larger than ours. However, Bilby and Ward (1989, 1991) found that LWD abundance decreased with increasing stream size for 2nd- to 5th-order streams in southwestern Washington. Lienkaemper and Swanson (1987) also reported decreasing LWD abundance with increasing stream size for five relatively undisturbed streams in Oregon. In contrast, Ralph et al. (1994) found no significant change with bank-full width or drainage area for unharvested streams in western Washington, and Bilby and Wasserman (1989) found no relationship with width for unharvested streams up to 20 m wide in the drier climate of eastern Washington. These different conclusions most likely reflect differences in drainage densities, flow regimes, geomorphic characteristics, or forest types among regions.

The position and orientation of LWD also changed with stream size, which influenced its role in pool formation. Fewer pieces spanned the channel perpendicular to flow (the position and orientation most associated with plunge and dammed pools) in larger streams, which explains why a lower percentage of LWD pieces formed pools there. Moreover, fewer pools were formed by single versus multiple LWD pieces in larger streams. Channel width exceeds most piece lengths in larger streams (Lienkaemper and Swanson 1987), which prevents pieces from anchoring at both stream banks, and the increased stream power more readily moves LWD. Robison and Beschta (1990*a*, 1990*b*) also found that less LWD spanned channels and was oriented perpendicular to flow in larger Alaskan streams than smaller ones. Bilby and Ward (1989) reported that LWD was oriented perpendicular to flow more often than expected in Washington streams <7 m wide, and was more often oriented diagonally downstream in streams >10 m wide.

Changes in the location and distribution of LWD with stream size also support the conclusion that larger streams

redistribute wood and alter its role in forming pools. Less wood occurred at least partially within the wetted channel and more occurred between the limits of the wetted and bank-full channels in larger streams, probably because the higher flows deposited it at the channel margins where it had little or no influence on fish habitat at base flow. The percentage of pieces wholly above the bank-full channel also decreased in larger streams, probably because channel width exceeded most debris length, which prevented logs from bridging stream banks. Robison and Beschta (1990*a*) also reported that the percentage of pieces wholly within the bank-full channel increased in larger Alaska streams, whereas those wholly above decreased.

LWD was often distributed relatively evenly in smaller old-growth streams, whereas clumps were always larger and more frequent than expected by chance in larger streams. Bisson et al. (1987) and Robison and Beschta (1990*a*) also reported that wood was relatively evenly distributed in 1st-, 2nd-, and small 3rd-order streams, where logs tended to lie where they fell because flows were generally inadequate to move them. Clumping in four of the seven smaller streams (two of which were the largest of the set) probably resulted from events occurring in the riparian forest (Swanson et al. 1976), such as one tree felled by wind also toppling several others. In contrast, flows in larger streams are able to redistribute more of the LWD and cause clumping. The study reach of Arapaho Creek, the largest stream, had two very large debris accumulations composed mainly of LWD >20 cm in diameter and >2 m long. Although some of these large accumulations stored large amounts of sediment, many were not associated with pools during base-flow. However, they may serve important functions at high flows, such as forming pools or shelter for fish, or dissipating stream energy that would otherwise cause erosion.

Low-gradient streams with more LWD had more overhead cover for fish provided by LWD and undercut banks. Bank materials in lower gradient streams are probably less resistant to erosion, and thus more prone to undercutting by flow deflected from LWD (Robison and Beschta 1990*b*). Woody debris may also stabilize upper portions of stream banks and allow undercutting below.

Characteristics of pools across streams

We found that the number of pools per 10 channel widths increased with higher LWD abundance in old-growth streams. Grette (1985) also reported a significant positive relationship between LWD and abundance of pools >10 m^2 in surface area in small low-gradient western Washington streams. In contrast, we found that pool frequency was not significantly related to total or bank-full LWD volume, which suggests that stable pieces within the bank-full channel that influence stream flow promote pool formation more than does overall debris volume. Andrus et al. (1988) and Fausch and Northcote (1992) also reported that the relatively few influential debris pieces that became stable accounted for most pool volume in two Pacific Northwest streams, and suggested that LWD volume was less important in pool formation than influential pieces.

The percentage of stream area as pools was higher in smaller streams that had more LWD, but was not related to either total or bank-full LWD volume. As for pool frequency, this suggests that influential pieces are more important than total or bank-full LWD volume in forming pools. In contrast, other investigators found no relationship between percentage pool area and LWD abundance (Carlson et al. 1990) or volume (Grette 1985) in Pacific Northwest streams.

Pool surface area and residual depth were also related more to stream size than to LWD size. Pools in larger streams were deeper and had larger surface area. However, after accounting for width and gradient among streams, there was no additional significant effect of LWD diameter, length, or volume on area or residual depth of pools formed by single pieces. These results suggest that geomorphic and fluvial characteristics exert primary control on individual pool size and type. Stream depth and gradient (i.e., stream power) and the resistance of bed and bank materials to erosion are important determinants of pool size (Wohl et al. 1993). Larger streams are capable of scouring deeper pools around LWD than smaller streams, all else being equal. Similarly, stable LWD will scour smaller or shallower pools in areas where cobble and boulder substrates predominate than in areas with fine substrates. Other researchers have also suggested that larger scale stream and valley characteristics such as gradient, bed-load composition, or geomorphic features may, beyond some threshold, override the influence of LWD in forming pools (Andrus et al. 1988; Evans et al. 1993).

There was no significant difference between pool frequency and residual depth in the disturbed and old-growth streams, even though disturbed streams had significantly less LWD, again suggesting that pool formation and size were not entirely dependent on LWD. Grette (1985) also found abundant pool habitat in Washington streams that had little LWD. However, the percent pool area was substantially less in three of four disturbed streams with low LWD abundance than in old-growth streams, suggesting that pools formed in disturbed streams had smaller surface areas than those formed by LWD, even though residual depths were similar.

Robison and Beschta (1990*b*) suggested that changing relationships between LWD and stream flow with stream size could influence pool types formed. We found that plunge and dammed pools were the most prevalent types formed by LWD, the majority of which were formed by pieces spanning the channel perpendicular to flow. Bilby and Ward (1989) reported the same findings for streams <7 m wide in southwestern Washington, but Bilby and Ward (1991) found more scour pools in larger streams. Fausch and Northcote (1992) found primarily plunge and dammed pools for reaches of a British Columbia stream with moderate gradient (1.5–4.1%), but found more lateral scour pools formed by LWD angled downstream in lower gradient reaches (≤1.2%). We also found that higher gradient streams had more plunge pools. Plunge pools at log steps dissipate stream energy (Heede 1972) so higher gradient streams have shorter distances between steps.

Implications for stream rehabilitation

Although LWD is smaller in the Rocky Mountain subalpine streams we studied than throughout the coastal region of the Pacific Northwest, its abundance and function

in forming pools and overhead cover for fish are similar. Thus, information gained from this research can be used by fishery managers in the Rocky Mountain region to simulate the LWD abundance, position, and orientation found in old-growth reaches when rehabilitating disturbed streams. However, it is also apparent from this study that LWD characteristics and function are significantly related to larger scale characteristics of stream size and gradient. Understanding the function of LWD in forming fish habitat in small mountain streams requires viewing the reach of interest at the drainage basin scale, because the role of LWD is largely determined by its location within the watershed. In addition, characteristics of individual pools formed by stable pieces of LWD, such as depth and surface area, are strongly influenced by characteristics of the watershed and stream itself, such as width, gradient, and the underlying geology. Knowledge of these larger scale relationships will allow managers interested in using LWD to increase pool habitat or overhead cover for fish to concentrate their efforts in stream reaches where success is most likely.

Acknowledgments

We thank U.S. Forest Service biologists Mike Young (Rocky Mountain Forest and Range Experiment Station), Nick Schmal (Medicine Bow National Forest), and Dave Gerhardt (Arapaho and Roosevelt national forests) for initiating the study and providing guidance and funding. Dennis Lowry, Carl Chambers, Steve Johnson, John Bustos, Lynne Bridgford, and Jim Nankervis of the Forest Service also assisted. Carrie Harrod, Lisa Schell, Ellie Jones, and volunteers Arlene Ganek, Mark Pearson, and Susan Madsen helped in the field. Phil Chapman and Ken Burnham gave advice on statistical analyses, and Gary White programmed the randomization test. Doris Rust drew the study area map. Ellen Wohl and Bob Behnke provided advice and guidance throughout. Mike Young, Bob Bilby, Mike Murphy, and an anonymous reviewer gave constructive criticism on earlier drafts.

References

Akaike, H. 1985. Prediction and entropy. A celebration of statistics. *Edited by* A.C. Atkinson and S.E. Fienberg. Springer, New York. pp. 1–24.

Alexander, R.R. 1980. Engelmann spruce – subalpine fir. Forest cover types of the United States and Canada. *Edited by* F.H. Eyre. Society of American Foresters, Washington, D.C. pp. 86–87.

Andrus, C.W., B.A. Long, and H.A. Froehlich. 1988. Woody debris and its contribution to pool formation in a coastal stream 50 years after logging. Can. J. Fish. Aquat. Sci. 45: 2080–2086.

Angermeier, P.L., and J.R. Karr. 1984. Relationship between woody debris and fish habitat in a small warmwater stream. Trans. Am. Fish. Soc. 113: 716–726.

Benke, A.C., R.L. Henry, III, D.M. Gillespie, and R.J. Hunter. 1985. Importance of snag habitat for animal production in southeastern streams. Fisheries, 10(5): 8–13.

Beschta, R.L., and W.S. Platts. 1986. Morphological features of small streams: significance and function. Water Resour. Bull. 22: 369–379.

Bilby, R.E., and G.E. Likens. 1980. Importance of organic debris dams in the structure and function of stream ecosystems. Ecology, 6: 1107–1113.

Bilby, R.E., and J.W. Ward. 1989. Changes in characteristics and function of woody debris with increasing size of streams in western Washington. Trans. Am. Fish. Soc. 118: 368–378.

Bilby, R.E., and J.W. Ward. 1991. Characteristics and function of large woody debris in streams draining old-growth, clear-cut, and second-growth forests in southwestern Washington. Can. J. Fish. Aquat. Sci. 48: 2499–2508.

Bilby, R.E., and L.J. Wasserman. 1989. Forest practices and riparian management in Washington State: data based regulation development. *In* Practical approaches to riparian resource management: an educational workshop. *Edited by* R.E. Gresswell, B.A. Barton, and J.L. Kershner. U.S. Department of the Interior, Bureau of Land Management, Billings, Mont. pp. 87–94.

Bisson, P.A., J.L. Nielsen, R.A. Palmason, and L.E. Grove. 1982. A system of naming habitat types in small streams, with examples of habitat utilization by salmonids during low streamflow. *In* Acquisition and utilization of aquatic habitat inventory information. Proceedings of a Symposium, Portland, Ore., Oct. 1981. *Edited by* N.A. Armatrout. Western Division, American Fisheries Society, Bethesda, Md. pp. 62–73.

Bisson, P.A., R.E. Bilby, M.D. Bryant, C.A. Dolloff, G.B. Grette, R.A. House, M.L. Murphy, K V. Koski, and J.R. Sedell. 1987. Large woody debris in forested streams in the Pacific Northwest: past, present, and future. *In* Proceedings of a symposium on Streamside Management: Forestry and Fisheries Interactions, University of Washington, Feb, 12–14, 1986. *Edited by* E.O. Salo and T.W. Cundy. University of Washington Institute of Forest Resources, Seattle, Wash. pp. 143–190.

Bryant, M.D. 1983. The role and management of woody debris in west coast salmonid nursery streams. N. Am. J. Fish. Manage. 3: 322–330.

Burnham, K.P., and D.R. Anderson. 1992. Data-based selection of an appropriate biological model: the key to modern data analysis. Wildlife 2001: populations. *Edited by* D.R. McCullough and R.H. Barrett. Elsevier, London. pp. 16–30.

Carlson, J.Y., C.W. Andrus, and H.A. Froehlich. 1990. Woody debris, channel features, and macroinvertebrates of streams with logged and undisturbed riparian timber in northeastern Oregon, U.S.A. Can. J. Fish. Aquat. Sci. 47: 1103–1111.

Chronic, H. 1980. Roadside geology of Colorado. Mountain Press Publishing Co., Missoula, Mont.

Evans, B.F., C.R. Townsend, and T.A. Crowl. 1993. Distribution and abundance of coarse woody debris in some southern New Zealand streams from contrasting forest catchments. N.Z. J. Mar. Freshwater Res. 27: 227–239.

Fausch, K.D., and T.G. Northcote. 1992. Large woody debris and salmonid habitat in a small coastal British Columbia stream. Can. J. Fish. Aquat. Sci. 49: 682–693.

Gill, J.L. 1978. Design and analysis of experiments in the animal and medical sciences. Vol. 2. Iowa State University Press, Ames, Iowa.

Grette, G.B. 1985. The role of large organic debris in juvenile salmonid rearing habitat in small streams. M.S. thesis, University of Washington, Seattle, Wash.

Harmon, M.E., J.F. Franklin, F.J. Swanson, P. Sollins, S.V. Gregory, J.D. Lattin, N.H. Anderson, S.P. Cline, N.G. Aumen, J.R. Sedell, G.W. Lienkaemper, K. Cromack, Jr., and K.W. Cummins. 1986. Ecology of coarse woody debris in temperate ecosystems. Adv. Ecol. Res. 15: 133–302.

Heede, B.H. 1972. Influences of a forest on the hydraulic geometry of two mountain streams. Water Resour. Bull. 8: 523–530.

Heede, B.H. 1985. Channel adjustments to the removal of log

steps: an experiment in a mountain stream. Environ. Manage. 9: 427–432.

Heifetz, J., M.L. Murphy, and K V. Koski. 1986. Effects of logging on winter habitat of juvenile salmonids in Alaskan streams. N. Am. J. Fish. Manage. 6: 52–58.

Hess, K., and R.R. Alexander. 1986. Forest vegetation of the Arapaho and Roosevelt National Forests in central Colorado: a habitat type classification. U.S. For. Serv. Res. Pap. No. RM-266.

Hogan, D. 1984. The influence of large organic debris on channel morphology in Queen Charlotte Island streams. Proc. Annu. Conf. West. Assoc. Fish Wildl. Agencies, 64: 263–273.

Hunt, R.L. 1993. Trout stream therapy. University of Wisconsin Press, Madison, Wis.

Johnson, S.W., J. Heifetz, and K V. Koski. 1986. Effects of logging on the abundance and seasonal distribution of juvenile steelhead in some southeastern Alaska streams. N. Am. J. Fish. Manage. 65: 532–537.

Keller, E.A., and F.J. Swanson. 1979. Effects of large organic material on channel form and fluvial processes. Earth Surf. Processes, 4: 361–380.

Lienkaemper, G.W., and F.J. Swanson. 1987. Dynamics of large woody debris in streams in old-growth Douglas-fir forests. Can. J. For. Res. 17: 150–156.

Lisle, T.E. 1987. Using "residual depths" to monitor pool depths independently of discharge. U.S. For. Serv. Res. Note No. PSW-394.

Long, B.A. 1987. Recruitment and abundance of large woody debris in an Oregon coastal stream system. M.Sc. thesis, Oregon State University, Corvallis, Ore.

Lowry, D.G. 1992. An old-growth forest inventory procedure for the Arapaho and Roosevelt National Forests, Colorado. *In* Old-growth forests in the Southwest and Rocky Mountain regions. U.S. For. Serv. Gen. Tech. Rep. No. RM-213. pp. 121–127.

Manly, B.F.J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, New York.

Murphy, M.L., and J.D. Hall. 1981. Varied effects of clear-cut logging on predators and their habitat in small streams of the Cascade Mountains, Oregon. Can. J. Fish. Aquat. Sci. 38: 137–145.

Murphy, M.L., and K V. Koski. 1989. Input and depletion of woody debris in Alaska streams and implications for streamside management. N. Am. J. Fish. Manage. 9: 427–436.

Murphy, M.L., J. Heifetz, S.W. Johnson, K V. Koski, and J.F. Thedinga. 1986. Effects of clear-cut logging with and without buffer strips on juvenile salmonids in Alaskan streams. Can. J. Fish. Aquat. Sci. 43: 1521–1533.

Nakamura, F., and F. J. Swanson. 1993. Effects of coarse woody debris on morphology and sediment storage of a mountain stream system in western Oregon. Earth Surf. Processes Landforms, 18: 43–61.

Orth, D.J. 1983. Aquatic habitat measurement. *In* Fisheries techniques. *Edited by* L.A. Nielsen and D.L. Johnson. American Fisheries Society, Bethesda, pp. 61–84.

Ralph, S.C., G.C. Poole, L.L. Conquest, and R.J. Naiman. 1994. Stream channel morphology and woody debris in logged and unlogged basins of western Washington. Can. J. Fish. Aquat. Sci. 51: 37–51.

Reeves, G.H., J.D. Hall, T.D. Roelofs, T.L. Hickman, and C.O. Baker. 1991. Rehabilitating and modifying stream habitats. Am. Fish. Soc. Spec. Publ. 19: 519–557.

Richmond, A.D. 1994. Characteristics and function of large woody debris in mountain streams of northern Colorado. M.S. thesis, Colorado State University, Fort Collins, Colo.

Riley, S.C., and K.D. Fausch. 1995. Trout population response to habitat enhancement in six northern Colorado streams. Can. J. Fish. Aquat. Sci. 52: 34–53.

Robison, E.G., and R.L. Beschta. 1990*a*. Characteristics of coarse woody debris for several coastal streams of southeast Alaska, U.S.A. Can. J. Fish. Aquat. Sci. 47: 1684–1693.

Robison, E.G., and R.L. Beschta. 1990*b*. Coarse woody debris and channel morphology interactions for undisturbed streams in southeast Alaska, U.S.A. Earth Surf. Processes Landforms, 15: 149–156.

Rosgen, D.L. 1985. A stream classification system. U.S. For. Serv. Gen. Tech. Rep. No. RM-120. pp. 91–95.

SAS Institute Inc. 1992. SAS/STAT guide for personal computers, version 7 edition. SAS Institute, Inc., Cary, N.C.

Strahler, A.N. 1957. Quantitative analysis of watershed geomorphology. Trans. Am. Geophys. Union, 38: 913–920.

Sullivan, K., T.E. Lisle, C.A. Dolloff, G.E. Grant, and L.M. Reid. 1987. Stream channels: the link between forests and fishes. *In* Proceedings of a Symposium on Streamside Management: Forestry and Fisheries Interactions, University of Washington, Feb. 12–14, 1986. *Edited by* E.O. Salo and T.W. Cundy. University of Washington Institute of Forest Resources, Seattle, Wash. pp. 39–97.

Swanson, F.J., G.W. Lienkaemper, and J.R. Sedell. 1976. History, physical effects, and management implications of large organic debris in western Oregon streams. U.S. For. Serv. Gen. Tech. Rep. No. PNW-56.

Tschaplinski, P.J., and G.F. Hartman. 1983. Winter distribution of juvenile coho salmon (*Oncorhynchus kisutch*) before and after logging in Carnation Creek, British Columbia, and some implications for overwinter survival. Can. J. Fish. Aquat. Sci. 40: 452–461.

White, R.J. 1975. Trout population responses to streamflow fluctuation and habitat management in Big Roche-a-Cri Creek, Wisconsin. Verh. Int. Ver. Theor. Angew. Limnol. 19: 2469–2477.

White, R.J. 1991. Resisted lateral scour in streams: its special importance to salmonid habitat and management. Am. Fish. Soc. Symp. 10: 200–203.

Wohl, E.E., K.R. Vincent, and D.J. Merritts. 1993. Pool and riffle characteristics in relation to channel gradient. Geomorphology, 6: 99–110.

Wroten, W.H. Jr., 1956. The railroad tie industry in the central Rocky Mountain region: 1867–1900. Ph.D. thesis, University of Colorado, Boulder, Colo.

Young, M.K., R.N. Schmal, and C.M. Sobczak. 1990. Railroad tie drives and stream channel complexity: past impacts, current status, and future prospects. *In* Proceedings of the Annual Meeting of the Society of American Foresters, Spokane, Wash., Sept. 24–27, 1989. Publication 89-02, Society of American Foresters, Bethedsa, Md. pp. 126–130.

Young, M.K., D. Haire, and M.A. Bozek. 1994. The effect and extent of railroad tie drives in streams of southeastern Wyoming. West. J. Appl. For. 9: 125–130.

Particulate light attenuation in a large subtropical lake

Karl E. Havens

Abstract: Deviations among Carlson's trophic state index values were used to quantify a 12-year history of seston composition and underwater light attenuation in Lake Okeechobee, Florida, U.S.A. Deviations between chlorophyll *a*, total phosphorus, and transparency-based trophic state indices indicated that (*i*) light attenuation is generally dominated by phosphorus-rich abiotic particles; (*ii*) abiotic light attenuation is maximal in a central lake region overlying soft mud sediments, and minimal in a near-littoral region overlying hard sand; and (*iii*) there has been a progressive increase in the relative contribution of algal pigments to total light attenuation between 1980 and 1992. Coincident with that 12-year trend, there have been declines in external nitrogen loads, lake water nitrogen:phosphorus ratios, and wind velocities. Surface water temperatures in the lake have significantly increased. Explanations for the trend in light attenuation include (*i*) more favorable meteorological conditions for algal growth, which increased the contribution of algae to overall light attenuation and (*ii*) reduced nitrogen:phosphorus ratios favoring proliferation of buoyant cyanobacteria, which are more effectively sampled by surface water monitoring. In either case, the trend did not coincide historically with enhanced nutrient loading, the common cause of algal proliferation in lakes.

Résumé : Pour quantifier l'évolution sur 12 ans de la composition du seston et de la diminution de la pénétration de la lumière dans le lac Okeechobee, en Floride (États-Unis), nous avons utilisé l'écart entre les valeurs de l'indice de l'état trophique de Carlson. Les écarts observés entre les indices établis d'après la teneur en chlorophylle *a*, le phosphore total et la transparence de l'eau dénotent que (*i*) avec la diminution de la pénétration de la lumière prédominent généralement les particules abiotiques riches en phosphore; (*ii*) la diminution abiotique de la pénétration de la lumière est maximum dans une région centrale du lac où le fond est vaseux et mou et elle est minimum dans une région proche du littoral où le fond est sableux et ferme; et (*iii*) la contribution des pigments d'algues à la diminution totale de la pénétration de la lumière a progressivement augmenté de 1980 à 1992. Parallèlement à cette tendance observée sur 12 ans, on a constaté la baisse des apports extérieurs d'azote, du rapport azote/phosphore dans l'eau du lac et de la vitesse des vents. La température des eaux superficielles a augmenté dans une mesure significative. La tendance à la diminution de la pénétration de la lumière est expliqué par les facteurs suivants : (*i*) les conditions météorologiques sont devenues plus favorables à la croissance des algues, de sorte que celles-ci ont contribué davantage à faire diminuer la pénétration de la lumière et (*ii*) la baisse du rapport azote/phosphore a favorisé la prolifération des cyanobactéries flottantes, organismes dont l'échantillonnage, effectué pour la surveillance des eaux superficielles, est plus efficace. En aucun cas, la tendance ne coïncide avec des apports supplémentaires de nutriments, cause la plus fréquente de la prolifération des algues dans les lacs.
[Traduit par la Rédaction]

Introduction

The degree of water column light penetration directly regulates the productivity of phytoplankton, benthic algae, and submerged macrophytes. That primary productivity, along with allochthonous inputs of organic carbon, sets the upper limit on secondary productivity at all higher trophic levels in the food web. Therefore, an understanding of factors that determine water column light penetration is important to the overall understanding of lake ecosystem dynamics.

The extent to which solar radiation is attenuated in the water column is a function of the light-absorbing properties of the water itself and the quantity and quality of dissolved and suspended materials. The intensity of light reaching a particular depth (I_z) is related to surface irradiance (I_o) in the following manner:

$$I_z = I_o e^{-(k_d)z}$$

The coefficient k_d represents the sum of attenuation coefficients for water, algal pigments, and dissolved and suspended abiotic materials.

Received July 27, 1994. Accepted March 2, 1995.
J12497

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In clear-water oligotrophic lakes, light attenuation is minimal because of a scarcity of particles and dissolved organic material in the water, and only at great depth does light limit the photosynthesis of planktonic and benthic algae. In hypereutrophic lakes, high concentrations of algal pigments may cause self-shading of underlying cells and light limitation (e.g., Straskraba 1980; Agusti et al. 1990). In shallow lakes with wind-driven sediment resuspension, light limitation may sometimes result from high concentrations of abiotic seston (Davalos et al. 1989).

An intensely studied case where both plankton and abiotic seston might contribute substantially to light attenuation is the shallow, subtropical Lake Okeechobee. The lake is the largest in the southeastern U.S.A. (1730 km^2), and with a mean depth of 2.7 m, it is one of the world's shallowest great lakes. The lake is eutrophic, and high concentrations of algal pigments are common (Phlips et al. 1993*a*). Owing to shallow depth and unconsolidated mud sediments (Olila and Reddy 1993), wind-driven sediment resuspension is a major regulator of both light penetration and nutrient concentrations (Maceina and Soballe 1990; Phlips et al. 1993*a*).

Recent studies in Lake Okeechobee (Havens 1994; Aldridge et al. 1995) documented a seasonal cycle where light limitation is maximal during winter and minimal during summer. Spatial patterns have also been elucidated: light-limited conditions are most common in the central region of the pelagic zone, and least common in a near-littoral western region (Phlips et al. 1993*a*). This zonation corresponds with the observed spatial distribution of fine sediment particles and turbidity (Carrick et al. 1994).

Phlips et al. (1995) described the relationships between algal biomass and transparency in Lake Okeechobee, and also considered the character of light attenuation and its seasonal variation. However, historical trends were not considered. Because Lake Okeechobee has undergone changes in water column nutrient concentrations, nutrient ratios, and algal biomass (Havens et al. 1995*a*), I hypothesized that there may have been changes in the nature of water column light attenuation. A long-term data base provided the opportunity to test this hypothesis. The relative contribution of phytoplankton and abiotic seston to light attenuation was quantified at four pelagic locations, for the period 1980–1992. The general approach was to compare trophic state indices (Carlson 1977) on the basis of measured concentrations of chlorophyll *a*, total phosphorus, and Secchi disk transparencies. This approach was previously used to determine the seasonal, spatial, and historic patterns of nutrient limitation in the lake (Havens 1994, 1995).

Description of the lake

Lake Okeechobee (Fig. 1) is a natural lake in South Florida, U.S.A., located at 27°00′N, 80°50′W. The lake is encircled by a flood control dike, constructed between the 1930s and 1960s, and hydrology is presently regulated by control structures on all but one inflow and on all outflows. Lake Okeechobee is turbid (Secchi depths average only 0.5 m) and naturally eutrophic (Stoermer et al. 1992), and it currently has total phosphorus concentrations averaging 87 $\mu g \cdot L^{-1}$, total nitrogen averaging 1.7 $mg \cdot L^{-1}$, and chlorophyll *a* averaging 25 $\mu g \cdot L^{-1}$. There is evidence of a post-1975 progression towards hypereutrophy (Janus et al. 1990; Havens et al. 1995*a*; Warren et al. 1995), and there recently have been cyanobacterial blooms over large areas of the pelagic zone (Jones 1987). The eutrophication trends are a concern because the lake is a drinking water supply for local municipalities, a wildlife habitat, and a source of irrigation water, and it supports an economically important fishery. In many ways, the limnological conditions and responses to human activities mirror those observed in shallow lake ecosystems around the world, including those in Japan, Mexico, and The Netherlands.

Methods

Sampling procedures

Data were obtained from the water quality data base of the South Florida Water Management District (SFWMD). All data were collected and processed according to consistent standard methods, as outlined in a comprehensive SFWMD quality assessment – quality control plan. This study used data from January 1980 to December 1992, when continuous monthly or semimonthly sampling was done for total phosphorus, chlorophyll *a*, and Secchi transparencies. Prior to 1980, chlorophyll *a* data were collected only in 1974 and 1975, at a low frequency relative to later years, and were analyzed using a different methodology.

Data were analyzed from four stations, representing north, east, south, and west quadrants of the pelagic zone (Fig. 1). At each station, transparencies were determined with a 20-cm black and white Secchi disk, and near-surface (0.25 m) samples were collected for total phosphorus and chlorophyll *a* analyses. Total phosphorus concentrations were determined colorimetrically after persulfate digestion in an autoclave (U.S. Environmental Protection Agency 1979). Chlorophyll *a* concentrations were determined spectrophotometrically according to Strickland and Parsons (1968), after filtering samples onto Whatman GF-C glass fiber filters (1.2-μm porosity), grinding in a tissue grinder, and overnight extraction in 90% acetone buffered with $MgCO_3$.

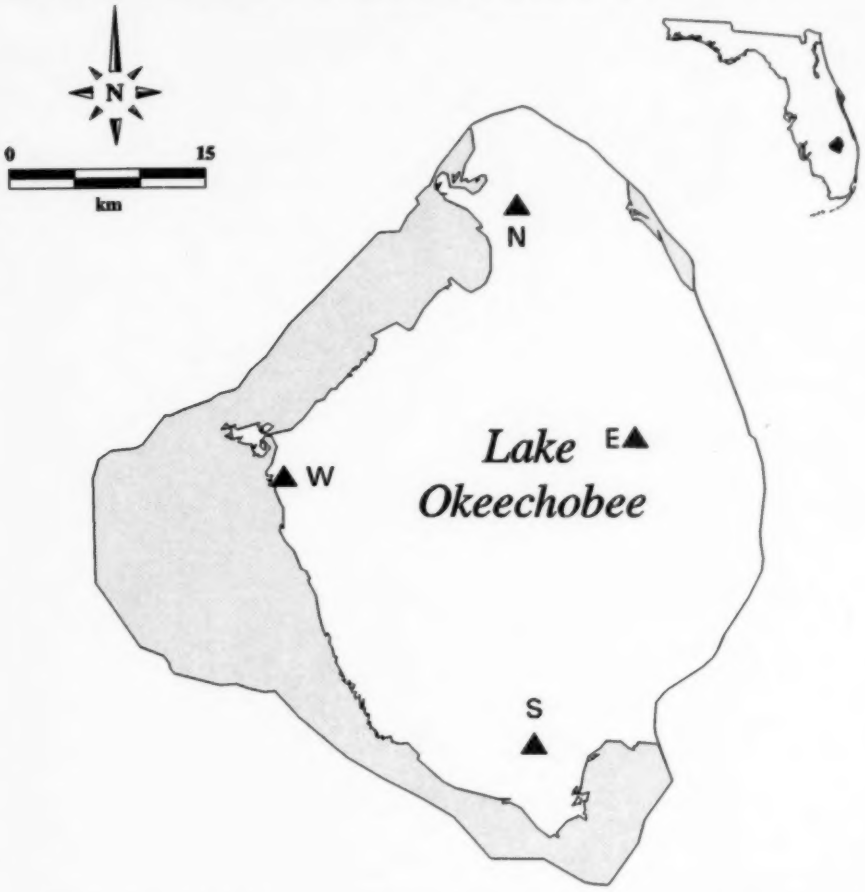
Trophic state index calculations

Trophic state indices (TSI) were calculated from monthly average Secchi disk transparencies (SD, m), total phosphorus concentrations (TP, $\mu g \cdot L^{-1}$), and chlorophyll *a* concentrations (CHL, $\mu g \cdot L^{-1}$) at the four lake stations, according to the equations given in Carlson (1977):

$$TSI(SD) = 10 \times (6 - \ln SD / \ln 2)$$
$$TSI(TP) = 10 \times (6 - \ln (48 / TP) / \ln 2)$$
$$TSI(CHL) = 10 \times (6 - (2.04 - 0.68 \ln CHL) / \ln 2)$$

The base index TSI(SD), from which the other two indices were derived, displays a 10-fold increase in value with each doubling of Secchi transparency. Extreme values (0 and 100) correspond to the transparency extremes (64 and 0.06 m, respectively) observed in nature. Carlson derived the TSI(TP) and TSI(CHL) indices from regression relationships between chlorophyll *a*, total phosphorus, and Secchi depths in a set of phosphorus-limited temperate lakes where algal pigments dominated light attenuation. In such lakes, the three index parameters display nearly equal values.

Fig. 1. Map of Lake Okeechobee and location in Florida, U.S.A. (inset). Locations of the four pelagic sampling stations are indicated: N, north; E, east; S, south; and W, west.



The trophic state index parameters have been widely used to quantify the degree of eutrophication of lakes. Some scientists and lake managers have proposed averaging the index values to obtain a single best estimate of trophic state (Kratzer and Brezonik 1982; Osgood 1982). However, this approach results in a loss of valuable information in cases where two or more of the index values differ. Carlson (1991) showed that differences among the index parameters (trophic state deviations) could provide information about both nutrient limitation and seston composition. Positive deviations of TSI(CHL) from TSI(TP) indicate phosphorus-limited conditions; negative deviations indicate limitation by some other factor, such as light, nitrogen, or zooplankton grazing. Havens (1994) used this approach to quantify the seasonal and spatial patterns of nutrient limitation in Lake Okeechobee, also including in the analysis a nitrogen TSI developed by Kratzer and Brezonik (1981). The limiting factors indicated by the TSI deviations were found to agree precisely with the results of nutrient bioassay experiments performed by Aldridge et al. (1995).

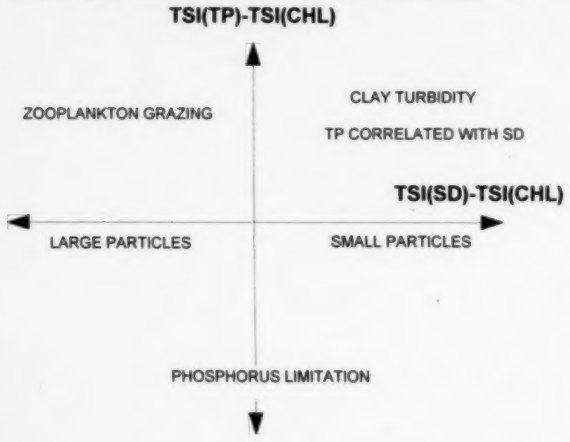
In the present study, I focused on the deviation between TSI(SD) and TSI(CHL), an indicator of seston composition. Carlson (1991) documented that when TSI(SD) < TSI(CHL) in temperate lakes, light attenuation is dominated by large particles, generally algal cells. When the deviation is positive, light attenuation is dominated by smaller particles, generally clay or silt. When the latter situation occurs, there is generally also a correlation between total phosphorus and transparency (Fig. 2).

This approach to quantifying seston composition has not been previously used in subtropical or tropical lakes, although Havens (1994) demonstrated that positive deviations of TSI(SD) from TSI(CHL) occurred during the windy winter months (when resuspension of mud bottom sediments is high) in Lake Okeechobee. During that season, Aldridge et al. (1995) and Phlips et al. (1993*a*) found widespread light limitation of algal growth as a result of high levels of abiotic seston.

Historic trends in trophic state indices and other parameters

Yearly mean values of the three trophic state indices and the deviations of TSI(SD) − TSI(CHL) were calculated, and historical trends were examined statistically with a

Fig. 2. Interpretation of deviations between Secchi depth (SD), total phosphorus (TP), and chlorophyll *a* (CHL) trophic state indices (TSIs), after Carlson (1991). Along the *x*-axis, deviations between TSI(SD) and TSI(CHL) indicate the size of seston particles. Negative deviations indicate large (algal) particles, and positive deviations indicate small (sediment) particles. Along the *y*-axis, deviations between TSI(TP) and TSI(CHL) indicate nutrient limitation, or lack thereof. Generally, data in the upper right quadrant (most data from Lake Okeechobee) indicate light-limited conditions owing to abiotic turbidity.



nonparametric Kendall's test (Hirsch et al. 1982). Statistical significance was defined as $p \leq 0.05$. Trends in several other variables, generally known to affect algal biomass and seston composition in shallow lakes, were also examined: surface water temperatures (measured in situ with a Hydrolab), lake water total and soluble nitrogen:phosphorus ratios, external nitrogen and phosphorus loading rates, and wind velocities.

Lake water total nitrogen:phosphorus ratios were based on total phosphorus concentrations, measured as described above, and total nitrogen concentrations, measured using standard nitrogen methodology for flow-injected autoanalyzers (U.S. Environmental Protection Agency 1987). Soluble nitrogen and phosphorus concentrations were measured on undigested samples, filtered in the field through 1.2-μm Poretics glass fiber primary filters and 0.45-μm Poretics polycarbonate secondary filters.

Nitrogen and phosphorus loads were calculated using the loading program FLUX (Walker 1983) modified for Lake Okeechobee, the measured nutrient concentrations in monthly or semimonthly water samples, and continuously gauged water flow rates. James et al. (1995) provide additional details. Daily wind velocities have been historically measured at a National Oceanic and Atmospheric Administration meteorological station located on the southwest shore of the lake.

Kendall's test was applied to the yearly averaged data to determine the statistical significance of any observed trends. In the case of in-lake parameters (surface temperature and nutrient ratios), the data were analyzed separately for north, east, south, and west sampling stations. All statistical analyses were done using SAS STAT (SAS Institute 1990).

Results and discussion

Lake-wide relationships

Combined data from the four pelagic stations illustrate the general relationships between the TSI values in Lake Okeechobee (Fig. 3). Correlations between yearly means of TSI(TP) and TSI(CHL) and between TSI(SD) and TSI(CHL) were not significant. However, there was a highly significant positive correlation between TSI(SD) and TSI(TP). Taken together, these results indicate that chlorophyll *a* is not a major contributor to underwater light attenuation, and that abiotic seston particles contain a relatively large fraction of the lake water total phosphorus.

These results are contrary to those obtained in deeper temperate lakes, where researchers have documented positive correlations between chlorophyll *a* concentrations and Secchi disk transparencies, and between chlorophyll *a* and total phosphorus (Carlson 1977, 1991; Nicholls and Dillon 1978; Prarie et al. 1989). The fact that shallow Lake Okeechobee deviates from this scenario has been previously noted. Phlips et al. (1995) examined contemporary (1988–1990) nutrient, chlorophyll, and transparency data, and Carrick et al. (1994) quantified the spatial distribution of chlorophyll and turbidity on the basis of satellite remote sensing. Phlips et al. (1995) documented an inverse correlation between chlorophyll and transparency, and Carrick et al. (1994) found that regions of chlorophyll and turbidity maxima were spatially segregated.

The high phosphorus content of abiotic light-attenuating particles in Lake Okeechobee also has been documented in studies of the sediment composition. Much of the lake bottom is composed of unconsolidated mud, with over 1500 $mg \cdot L^{-1}$ total phosphorus, mostly associated with particles rather than pore water (Olila and Reddy 1993).

Trends at four pelagic stations

Recent studies have demonstrated that physical, chemical, and biological conditions in Lake Okeechobee exhibit great spatial heterogeneity. Phlips et al. (1993*a*) documented four statistically distinct pelagic ecological zones, on the basis of a discriminant function analysis of light, chlorophyll *a*, and nutrient data. The historical trends in TSIs and their deviations were therefore considered separately for the four pelagic locations.

There was a consistent interstation variability in TSI(SD) values over the historical period (Fig. 4). At the east station, TSI(SD) values generally have exceeded 70, indicating hypereutrophy, a trophic misclassification (sensu Carlson 1991), since chlorophyll *a* concentrations indicated eutrophic conditions. The east station is located within a large central mud-bottomed region of the lake, where sediment resuspension is frequent, and where light penetration is greatly reduced. In contrast, the west station generally had the lowest TSI(SD) values, although they also indicated eutrophy. This west station is located near the littoral fringe, a region that is underlain by sand sediments and supports a dense benthic plant assemblage (Phlips et al. 1993*b*). It recently has been determined that greater wind velocities are required to resuspend sediments at this region, relative to

Fig. 3. Relationships between (A) TSI(SD) and TSI(CHL), (B) TSI(CHL) and TSI(TP), and (C) TSI(SD) and TSI(TP). The index parameters are defined in the caption for Fig. 2. Results of Kendall's Tau-b correlation analyses are indicated.

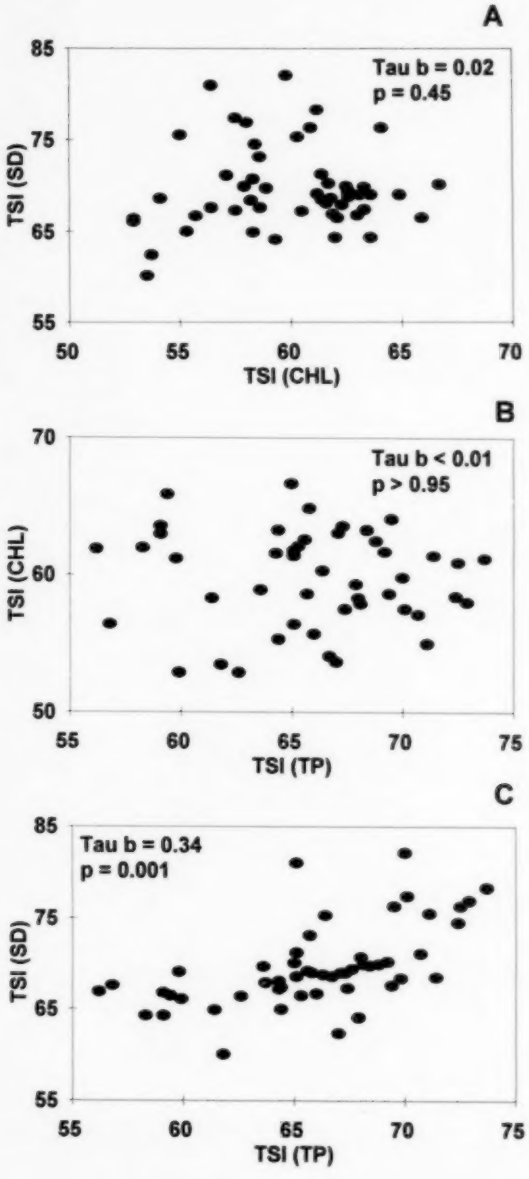
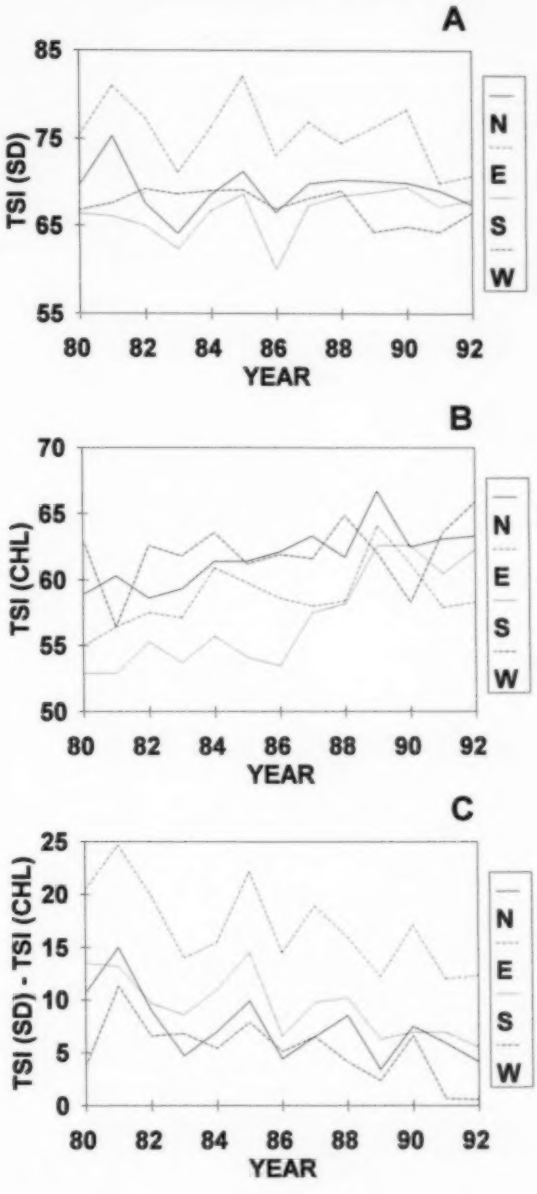


Fig. 4. Historical trends in (A) TSI(SD), (B) TSI(CHL), and (C) the deviation of TSI(CHL) from TSI(SD). The index parameters are defined in the caption for Fig. 2.



the mud-bottomed lake center (C. Hanlon, South Florida Water Management District, personal communication). The two other sampling stations, in the north and south, occur near the periphery of the mud-bottomed zone, and their limnological state is affected to an intermediate degree by resuspended sediments.

There were no statistically significant historical trends in TSI(SD), except at the south station, where TSI(SD) has increased (Table 1) indicating that the water has become less transparent.

The TSI(CHL) values have historically been lowest at the south station and highest at the north and west stations. The north and west stations occur in regions where there is suitable light penetration for algal photosynthesis (Phlips

Table 1. Kendall's Tau-b rank correlation coefficients and levels of statistical significance associated with the relationships between sampling year and the three trophic state variables, TSI(SD), TSI(CHL), and TSI(SD) − TSI(CHL), as defined in the text.

	Location			
Dependent variable	North	East	West	South
TSI(SD)	−0.05	−0.25	−0.33	+0.41
	0.81	0.22	0.11	0.05
TSI(CHL)	+0.72	+0.39	+0.21	+0.67
	0.01	0.07	0.33	0.01
TSI(SD) − TSI(CHL)	−0.49	−0.49	−0.50	−0.54
	0.02	0.02	0.01	0.01

Table 2. Kendall's Tau-b rank correlation coefficients and levels of statistical significance associated with the relationships between sampling year and six limnological variables known to affect algal biomass and (or) light attenuation in shallow lakes.

	Location				
Dependent variable	North	East	West	South	Lake-wide
Water temperature	+0.51	+0.17	+0.59	+0.67	
	0.01	0.39	0.01	0.01	
Total N:P ratio	−0.41	−0.51	−0.18	−0.36	
	0.05	0.01	0.39	0.09	
Soluble N:P ratio	+0.23	−0.26	+0.05	−0.28	
	0.27	0.22	0.81	0.18	
Wind velocity					−0.34
					0.09
Total P loading rate					−0.08
					0.71
Total N loading rate					−0.39
					0.07

et al. 1993*a*), and they are also locations of locally high nutrient inputs (Fluck et al. 1992) and intense and frequent algal blooms (Carrick et al. 1994; Havens et al. 1995*b*).

There were statistically significant increases in TSI(CHL) at three of the four stations during the historical period (Table 1). In the north and east, there was a gradual upward trend, but in the south, the increase occurred rapidly between 1986 and 1990. There was considerably less interstation variability in TSI(CHL) after 1988 than during the previous years. That is, the different regions of the lake have become more homogeneous in terms of their chlorophyll *a*-based trophic states.

From the standpoint of lake users, eutrophication trends at the south end of the lake are a particular concern, because that region is heavily utilized for recreational fishing. It is also an area of extensive macrophytes, which support a diverse assemblage of macroinvertebrates (Warren et al. 1995), fish (Bull et al. 1995), and wading birds (J.P. Smith et al. 1995). Therefore, algal proliferation in this region is a greater threat to ecosystem integrity than in the relatively species-poor central mud-bottomed region of the lake.

Trophic state deviations, TSI(SD) − TSI(CHL), have historically been positive at all stations, indicating that small abiotic particles dominate underwater light attenuation. However, the magnitude of the deviations has varied spatially. Deviations were most positive at the east station, and least positive at the north and west stations, indicating the least and greatest contributions of algal pigments to light attenuation, respectively. This spatial pattern is consistent with the results of other studies. Carrick et al. (1994), in particular, found that maximal concentrations of chlorophyll a occurred in the extreme northern end of the lake, near major tributaries draining agricultural areas, and to the west, near the littoral zone. They attributed this pattern to localized stimulation of algal growth by external nutrient inputs. Others have noted (Phlips et al. 1993*a*) that these same regions have underwater light levels conducive to algal photosynthesis, and that spatial variability may instead reflect a suppression of algal growth as a result of light limitation in mid-lake (Havens 1994; Aldridge et al. 1995). Several studies have documented that the mid-lake region displays the maximal levels of abiotic turbidity (Phlips et al. 1993*a*; Carrick et al. 1994; Phlips et al. 1995), and it appears that the critical forcing function is wind velocity (Maceina and Soballe 1990), which determines wave height, bottom shear stress, and ultimately, sediment resuspension.

There have been statistically significant historical declines in TSI(SD) − TSI(CHL) at all four stations (Table 1). At the west station, the 1991 and 1992 TSI deviations were near zero, indicating light attenuation dominated by algae according to Carlson (1991).

This study is the first to describe historical trends in seston composition and light attenuation in Lake Okeechobee. However, a study of contemporary data by Phlips et al. (1995) provides comparable results. Phlips et al. (1995) measured Secchi depths, chlorophyll *a* concentrations, and water column light fluxes (determined directly with a submersible quantum light probe) during 1988–1990 at four pelagic sites (north, south edge, central, and west). The north and west stations are the same as those considered here. They found significant inverse relationships between chlorophyll *a* concentrations and Secchi depths at the south and west stations, both located near the littoral fringe in areas of firm sediments. In contrast, chlorophyll *a* and Secchi depth were not correlated in the north, but were positively correlated in the central mud zone. The maximal contribution of chlorophyll *a* to total light attenuation occurred at the west and south stations, and the minimal contribution occurred in the lake center. Temporal trends were not considered, given the short duration (3 years) of the study.

Many authors have commented on the difficulty in assigning a trophic status to a lake where index values differ. Phlips et al. (1995) suggested that direct measurements of water column light flux are essential to resolving the "sometimes confounding trophic implications of widely utilized parameters such as Secchi disk depth, TP, and chlorophyll." While their results clearly demonstrate the

value of measuring light flux, I would argue that the more widely utilized parameters, and especially comparisons among them, are "informational" rather than "confounding." In the case of Lake Okeechobee, they have permitted the determination of lake-wide nutrient limitation (Havens 1994), and in the present study, the nature of underwater light attenuation. Using such routine measures, considerable information can be obtained at little cost and effort beyond the scope of typical lake monitoring programs.

Dissolved organic color, not considered here, also can affect the TSI(SD) − TSI(CHL) deviation. High levels may cause positive deviations and lead to the incorrect conclusion that abiotic particles are attenuating light. Therefore, it is necessary to determine (or at least estimate) the influence of color in any study such as this. It was recently documented (Limno-Tech 1993) that in Lake Okeechobee, organic color is quite low, most often below 40 $mg \cdot L^{-1}$ Pt. At this level, color does not markedly affect the relationship between Secchi depth and chlorophyll *a* concentration (Carlson 1991).

Trends in other variables and correlational relationships

External nutrient loading rates control algal biomass and productivity in many freshwater lakes (Sas et al. 1989). In shallow lakes, where sediment resuspension can result in high internal nutrient loading, wind velocity also has the potential to affect seston composition, light penetration, and ultimately algal production (Maceina and Soballe 1990). This is especially the case where unconsolidated mud sediments predominate, as in Lake Okeechobee.

I considered the 12-year trends in each of these variables, as well as surface water temperatures and nitrogen:phosphorus ratios (Table 2), to elucidate potential causes for the upward trends in algal biomass indicated by increases in TSI(CHL), and the increased importance of algal pigments in total underwater light attenuation, indicated by declines in TSI(SD) − TSI(CHL).

Contrary to the TSI trends, which suggest an algal response to some growth-stimulating factor, there have recently been declines in external nutrient loads. When considering the yearly averages, only the nitrogen loading trend was marginally significant. James et al. (1995) considered monthly data and found significant declines in flow-weighted input concentrations of both nitrogen and phosphorus between 1981 and 1992, following significant increases in input concentrations between 1973 and 1980. Hence, the 1980 to 1992 eutrophication trend in TSI(CHL) has followed, rather than coincided with, increased nutrient inputs.

Although it is possible that historic high nutrient inputs have established conditions that are favorable for algal proliferation, some other factor(s) appear(s) to be the cause of the recent TSI trends. During the 12-year period considered here, three variables (water temperature, wind velocity, and total nitrogen:phosphorus ratio) displayed trends consistent with the promotion of algal growth. Surface water temperatures increased by 15%, from 23°C in the early 1980s to 26°C in the early 1990s. The trend was statistically significant at the south and west stations. Wind velocities decreased by approximately 20%, from an average of 3.0 $km \cdot h^{-1}$ in the early 1980s to near 2.5 $km \cdot h^{-1}$ in the early 1990s. The historic trend was marginally significant. Lake-wide total nitrogen:phosphorus ratios averaged 34:1 in the early 1980s and 20:1 in the early 1990s. The trend was statistically significant at the north and east stations, and marginally significant at the south station.

On the basis of these results, four hypotheses are proposed that can be tested in future experimental research.

Hypothesis 1. Algal growth has been stimulated by enhanced water temperatures.

Under conditions of nutrient sufficiency, a 3°C rise in water temperature might be expected to cause a 20–30% increase in algal growth rates, assuming a Q_{10} of between 2.0 and 2.3 (Reynolds 1993). This estimate is within the range of chlorophyll *a* concentration increases documented by Havens et al. (1995*b*) at the various lake stations, and reflected by the TSI(CHL) increases described here. Further, there is support for the notion that nutrients are in surplus. Soluble reactive phosphorus levels are rarely below the limits of detection (2 $\mu g \cdot L^{-1}$), and during nutrient bioassays performed between 1989 and 1992, there were never primary growth responses to phosphorus additions (Aldridge et al. 1995). Although there were growth responses to nitrogen additions, the dominant algal taxa in the lake have the capacity to fix atmospheric nitrogen, a potentially unlimited source (Phlips and Ihnat 1995).

Nevertheless, if the temperature hypothesis were correct, the most pronounced trends in TSI(CHL) should have occurred at the locations of greatest temperature change. The former occurred in the north and south; the latter occurred in the south and west. These results are not entirely consistent with the hypothesis.

Also, one cannot rule out the possibility that the historic trend in surface water temperature is due to, rather than the cause of, enhanced algal biomass in the water column. Previous studies have shown that by attenuating downwelling solar radiation, algal particles can influence not only the light conditions, but also the thermal profile of lakes (Mazumder et al. 1990). However, one might expect that the phenomenon observed by those researchers, in a strongly stratified temperate lake, might be disrupted by the frequent (daily) mixing of the water column in shallow wind-driven Lake Okeechobee.

Hypothesis 2. Algal growth has been stimulated by greater underwater light penetration.

In Lake Okeechobee, it has been documented that wind-induced sediment resuspension is an important factor affecting water column turbidity (Maceina and Soballe 1990), and that where abiotic turbidity is high, the phytoplankton may become light limited (Havens 1994; Aldridge et al. 1995). Therefore, a substantial decline in wind velocity might result in enhanced algal growth, mediated by greater underwater light penetration. Between the early 1980s and 1990s, there was a consistent decline in average wind velocities measured at Lake Okeechobee; overall velocities changed by 15% (from 4.8 to 4.0 $km \cdot h^{-1}$). The downward trend in the TSI(SD) − TSI(CHL) deviation coincided with this change, supportive of the hypothesis because the deviation trend suggests a decline in the relative contribution of resuspended sediment particles to light attenuation. However, a 0.8 $km \cdot h^{-1}$ decline in wind velocities is very

low in relation to daily variability (from 0 to over 15 $km \cdot h^{-1}$). Hence, the evidence in support of the wind hypothesis is also equivocal.

Hypothesis 3. Lower nitrogen:phosphorus ratios have favored buoyant cyanobacteria, and this has resulted in greater surface chlorophyll a *concentrations.*

It has recently been documented (Cichra et al. 1995) that the relative biovolume of cyanobacteria increased between the late 1970s and the early 1990s in Lake Okeechobee. V.H. Smith et al. (1995) concluded that the trend occurred because of the precipitous decline in lake-wide nitrogen: phosphorus ratios. Low ratios have previously been shown to facilitate cyanobacterial dominance in temperate and tropical lake plankton assemblages (Smith 1986), and the present ratios in Lake Okeechobee (near 20:1) are considerably lower than the critical level at which this transition is thought to occur (Smith 1983). Because several of the dominant cyanobacterial taxa in the lake, including the frequent bloom-former *Anabaena circinalis*, have the capacity to form gas vacuoles and rise to the water surface (Reynolds 1987), surface chlorophyll *a* concentrations may become elevated when they dominate. Because the South Florida Water Management District collects chlorophyll *a* samples at the water surface, this change in algal composition might account for the TSI(CHL) increase reported here. Averaged over the entire water column, chlorophyll *a* concentrations might not have changed. We do not have a historic record of subsurface data to test this.

Hypothesis 4. Lower nitrogen:phosphorus ratios have favored dominance by large cyanobacteria, which are not effectively exploited by zooplankton herbivores.

The zooplankton in Lake Okeechobee is presently dominated by small rotifers, especially *Keratella* spp. (Crisman et al. 1995), which do not exploit filamentous cyanobacteria (Havens 1993). On the basis of results from several Florida lakes with similar plankton composition, Crisman et al. (1995) concluded that the zooplankton of Lake Okeechobee do not exert significant grazing pressure on the phytoplankton. We are presently testing this hypothesis with experimental manipulations and direct measurements of zooplankton grazing. There is not a historic record of zooplankton grazing, but the results from an experimental manipulation performed in the late 1970s, when diatoms were more dominant in the phytoplankton, lends support to the notion that grazing impacts were previously important. Brezonik et al. (1979) reduced the densities of resident zooplankton in large limnocorrals by adding planktivorous fish. In response, there was a great increase in algal biomass, suggesting top-down control.

Taken together, these empirical results and the hypotheses generated from them provide a direction for future experimental and process-oriented research to explain the paradoxical increases in chlorophyll and algal light attenuation that have coincided with declines, not increases, in nutrient inputs to the lake. In general, the present results suggest a degree of instability in the phytoplankton of this large lake, where historic nutrient overloading has created conditions of phosphorus surplus, and where the dominant cyanobacteria may respond to unpredictable changes in the environment (perhaps higher temperatures and (or) lower wind velocities) that favor their growth. Interestingly, a similar situation has recently occurred in the eutrophic Lake Kinneret, Israel, where nitrogen:phosphorus ratios have declined from 41–87 in the early 1980s to 20–32 after 1981 (Gophen 1994). In that case, blooms of a nitrogen fixer, *Aphanizomenon ovalisporum*, have also become very abundant.

Acknowledgements

This paper was enhanced by suggestions from K. Cummins, S. Gray, C. Hanlon, R.T. James, and two anonymous reviewers.

References

Agusti, S., C.M. Duarte, and D.E. Canfield, Jr. 1990. Phytoplankton abundance in Florida lakes: evidence for the frequent lack of nutrient limitation. Limnol. Oceanogr. 35: 181–188.

Aldridge, F.J., E.J. Phlips, and C.L. Schelske. 1995. The use of nutrient enrichment bioassays to test for spatial and temporal distribution of limiting factors affecting phytoplankton dynamics in Lake Okeechobee, Florida. Ergeb. Limnol. 45: 177–190.

Brezonik, P.L., E.C. Blancher, V.B. Myers, C.L. Hilty, M.K. Leslie, C.R. Kratzer, G.D. Marbury, B.R. Snyder, T.L. Crisman, and J.J. Messer. 1979. Factors affecting primary production in Lake Okeechobee, Florida. Report to the Florida Sugar Cane League, Clewiston, Fla.

Bull, L.A., D.D. Fox, D.W. Brown, L.J. Davis, S.J. Miller, and J.G. Wullschleger. 1995. Fish distribution in limnetic areas of Lake Okeechobee, Florida. Ergeb. Limnol. 45: 333–342.

Carlson, R.E. 1977. A trophic state index for lakes. Limnol. Oceanogr. 22: 361–369.

Carlson, R.E. 1991. Expanding the trophic state concept to identify non-nutrient limited lakes and reservoirs. pp. 59–71. *In* Enhancing the states' lake management programs. U.S. Environmental Protection Agency, Washington, D.C.

Carrick, H.J., D. Worth, and M.L. Marshall. 1994. The influence of water circulation on chlorophyll–turbidity relationships in Lake Okeechobee as determined by remote sensing. J. Plankton Res. 16: 1117–1135.

Cichra, M.F., S. Badylak, N. Henderson, B.H. Reuter, and E.J. Phlips. 1995. Phytoplankton community structure in the open water zone of a shallow subtropical lake (Lake Okeechobee, Florida, USA). Ergeb. Limnol. 45: 157–175.

Crisman, T.L., E.J. Phlips, and J.R. Beaver. 1995. Zooplankton seasonality and trophic state relationships in Lake Okeechobee, Florida. Ergeb. Limnol. 45: 213–232.

Davalos, L., O.T. Lind, and R.D. Doyle. 1989. Evaluation of phytoplankton-limiting factors in Lake Chapala, Mexico: turbidity and the spatial and temporal variation in algal assay response. Lake Reservoir Manage. 5: 99–104.

Fluck, R.C., C. Fonyo, and E. Flaig. 1992. Land use based phosphorus balances for Lake Okeechobee, Florida, drainage basins. Appl. Eng. Agric. 8: 813–820.

Gophen, M. 1994. Blue-green algae and Lake Kinneret. ASLO Bull. 3: 5.

Havens, K.E. 1993. An experimental analysis of macrozooplankton, microzooplankton, and phytoplankton interactions in a temperate eutrophic lake. Arch. Hydrobiol. 127: 9–20.

Havens, K.E. 1994. Seasonal and spatial variation in nutrient limitation in a shallow subtropical lake (Lake Okeechobee, Florida) as evidenced by trophic state index deviations. Arch. Hydrobiol. 131: 39–53.

Havens, K.E. 1995. Secondary nitrogen limitation in a subtropical lake impacted by non-point source agricultural pollution. Environ. Pollut. 89: 241–246.

Havens, K.E., V.J. Bierman, E.G. Flaig, C. Hanlon, R.T. James, B.L. Jones, and V.H. Smith. 1995*a*. Historical trends in the Lake Okeechobee ecosystem. VI. Synthesis. Arch. Hydrobiol. Suppl. Monogr. Beitr. 107: 99–109.

Havens, K.E., C. Hanlon, and R.T. James. 1995*b*. Seasonal and spatial variation in algal bloom frequencies in Lake Okeechobee, Florida, USA. Lake Reservoir Manage. 10: 139–148.

Hirsch, R.M., J.R. Slack, and R.A. Smith. 1982. Techniques of trend analysis for monthly water quality data. Water Resour. Res. 18: 107–121.

James, R.T., B.L. Jones, and V. Smith. 1995. Historical trends in the Lake Okeechobee ecosystem. II. Nutrient budgets. Arch. Hydrobiol. Suppl. Monogr. Beitr. 107: 25–47.

Janus, L.L., D.M. Soballe, and B.L. Jones. 1990. Nutrient budget analyses and phosphorus loading goal for Lake Okeechobee, Florida. Verh. Int. Ver. Theor. Angew. Limnol. 24: 538-546.

Jones, B.L. 1987. Lake Okeechobee eutrophication research and management. Aquatics (Tallahassee, Fla.), 9: 21–26.

Kratzer, C.R., and P.L. Brezonik. 1981. A Carlson-type trophic state index for nitrogen in Florida lakes. Water Resour. Bull. 17: 713–715.

Kratzer, C.R., and P.L. Brezonik. 1982. Reply to discussion by Richard Osgood, "A Carlson-type trophic state index for nitrogen in Florida lakes." Water Resour. Bull. 18: 543–544.

Limno-Tech. 1993. Preliminary assessment of nitrogen impacts on the Lake Okeechobee Ecosystem. Report to the South Florida Water Management District, West Palm Beach, Fla.

Maceina, M.J., and D. Soballe. 1990. Wind-related limnological variation in Lake Okeechobee, Florida. Lake Reservoir Manage. 6: 93–100.

Mazumder, A., W.D. Taylor, D.J. McQueen, and D.R.S. Lean. 1990. Effects of fish and plankton on lake temperature and mixing depth. Science (Washington, D.C.), 247: 312–315.

Nicholls, K.H., and P.J. Dillon. 1978. An evaluation of phosphorus–chlorophyll–phytoplankton relationships for lakes. Int. Rev. Gesamten. Hydrobiol. 63: 141–145.

Olila, O.G., and K.R. Reddy. 1993. Phosphorus sorption characteristics of sediments in shallow eutrophic lakes of Florida. Arch. Hydrobiol. 129: 45–65.

Osgood, R.A. 1982. Using differences among Carlson's trophic state index values in regional water quality assessment. Water Resour. Bull. 18: 67–74.

Phlips, E.J., and J. Ihnat. 1995. Planktonic nitrogen fixation in a shallow subtropical lake, Lake Okeechobee, Florida, U.S.A. Ergeb. Limnol. 45: 191–201.

Phlips, E.J., F.J. Aldridge, P. Hansen, P.V. Zimba, J. Ihnat, M. Conroy, and P. Ritter. 1993*a*. Spatial and temporal variability of trophic state parameters in a shallow subtropical lake (Lake Okeechobee, Florida, U.S.A.). Arch Hydrobiol. 128: 437–458.

Phlips, E.J., P.V. Zimba, M. Hopson, and T.L. Crisman. 1993*b*. Dynamics of the plankton community in submerged plant dominated regions of Lake Okeechobee, Florida, USA. Verh. Int. Ver. Theor. Angew. Limnol. 25: 423–426.

Phlips, E.J., F.J. Aldridge, C.L. Schelske, and T.L. Crisman. 1995. Relationships between Secchi disk depth, light availability and chlorophyll *a* in a large shallow sub-tropical lake. Limnol. Oceanogr. 40: 416–421.

Prarie, Y.T., C.M. Duarte, and J. Kalff. 1989. Unifying nutrient–chlorophyll relationships in lakes. Can. J. Fish. Aquat. Sci. 46: 1176–1182.

Reynolds, C.S. 1987. Cyanobacterial water blooms. Adv. Bot. Res. 13: 67–143.

Reynolds, C.S. 1993. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge, U.K.

Sas, H., I. Ahlgren, H. Bernhardt, B. Bostrom, J. Clasen, C. Forsberg, D. Imboden, L. Kamp-Nielsen, L. Mur, N. deOude, C. Reynolds, H. Schreurs, K. Seip, U. Sommer, and S. Vermij. 1989. Lake restoration by reduction of nutrient loading: expectations, experiences, extrapolation. Academia-Verlag, Germany.

SAS Institute. 1990. SAS user's guide: statistics, version 6 edition. SAS Institute Inc., Cary, N.C.

Smith, J.P., J.R. Richardson, and M.W. Callopy. 1995. Foraging habitat selection among wading birds at Lake Okeechobee, Florida in relation to hydrology and vegetative cover: a broad overview. Ergeb. Limnol. 45: 247–285.

Smith, V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science (Washington, D.C.), 221: 669–671.

Smith, V.H. 1986. Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton. Can. J. Fish. Aquat. Sci. 43: 148–153.

Smith, V.H., V.J. Bierman, B.L. Jones, and K.E. Havens. 1995. Historical trends in the Lake Okeechobee ecosystem. IV. Nitrogen:phosphorus ratios, cyanobacterial dominance, and nitrogen-fixation potential. Arch. Hydrobiol. Suppl. Monogr. Beitr. 107: 69–86.

Stoermer, E.F., N.A. Andersen, and C.L. Schelske. 1992. Diatom succession in the recent sediments of Lake Okeechobee, Florida, USA. Diatom Res. 7: 367–386.

Straskraba, M. 1980. The effect of physical variables on freshwater production: analysis based on models. *In* The functioning of freshwater ecosystems. *Edited by* E.D. LeCren and L. McConnell. Cambridge University Press, Cambridge, U.K. pp. 13–84.

Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. No. 167.

U.S. Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. U.S. Environmental Protection Agency, Washington, D.C.

U.S. Environmental Protection Agency. 1987. Handbook of methods for acid deposition studies. U.S. Environmental Protection Agency, Washington, D.C.

Walker, W.W., Jr. 1983. FLUX: a computer program for estimating mass discharges. Prepared for the Chief of Engineers, Waterways Experiment Station, United States Army Corps of Engineers, Vicksburg, Miss.

Warren, G.L., M.J. Vogel, and D.D. Fox. 1995. Distribution and trophic status of Lake Okeechobee limnetic zone benthic invertebrate communities. Ergeb. Limnol. 45: 317–332.

Optimal sampling period for predicting phosphorus and chlorophyll concentrations in north-temperate lakes

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Abstract: Because total phosphorus and chlorophyll *a* concentrations vary seasonally within north-temperate lakes, estimates of lake trophic status are usually based on several to many sampling visits. However, because such effort is not always logistically possible, the empirical relationships developed between these trophic status indicators may depend upon seasonal patterns of sampling. Clearly, it is desirable to know if some periods of the year are more likely to produce representative estimates of total phosphorus and chlorophyll *a*, or, alternatively, if sampling in other periods should be restricted or even avoided. Data from north-temperate lakes are used to examine the degree to which single monthly samples for total phosphorus and chlorophyll *a* represent seasonal means. Analyses indicate that the least precise and least accurate estimates of the means occur when single-visit synoptic surveys are conducted during April. Not until August to September do single sampling visits provide data that approximate those represented by the means garnered through more frequent sampling.

Résumé : Puisque les concentrations de phosphore total et de chlorophylle *a* varient avec le passage des saisons dans les lacs de la zone tempérée boréale, on fonde ordinairement les estimations de l'état trophique des lacs sur les résultats de plusieurs relevés à des nombreux relevés sur le terrain. Cependant, et comme il n'est pas toujours possible de procéder de façon aussi complète pour des raisons de logistique, il se peut que les relations empiriques fondées sur ces indicateurs de l'état trophique varient en fonction du régime saisonnier d'échantillonnage. De toute évidence, il est souhaitable de déterminer si certaines périodes de l'année sont plus propices que d'autres au calcul d'estimations représentatives du phosphore total et de la chlorophylle *a* ou encore si l'échantillonnage devrait être limité ou même évité à certaines autres périodes. On se sert de données obtenues dans des lacs de la zone tempérée boréale pour examiner dans quelle mesure des échantillons mensuels uniques correspondent bien aux moyennes saisonnières. Ces analyses nous indiquent que les estimations les moins précises et les moins exactes des moyennes sont produites lorsque les prélèvements synoptiques sont faits au cours de virées uniques en avril. Ce n'est pas avant la période d'août–septembre que ces virées uniques fournissent des résultats s'approchant de ceux représentés par les moyennes établies au moyen de prélèvements plus fréquents.

[Traduit par la Rédaction]

Introduction

Variation in chlorophyll *a* (CHL*a*) and total phosphorus (TP) concentrations over the growing season in north-temperate lakes often exceeds the variation measured among sites or years (e.g., El Shaarawi and Kwiatkowski 1977; Heyman et al. 1984; Knowlton et al. 1984; Kwiatkowski 1985; Hanna and Peters 1991). As a result, mean values may be severely biased if based on fewer than five samples collected during either the season or year (Marshall et al. 1988; France and Peters 1992). However, logistic or financial constraints often preclude intensive sampling such that synoptic surveys may involve visiting lakes only once during a survey (e.g., Environmental Protection Agency 1978; Paulsen 1992). Given the marked seasonal variation in water quality variables (e.g., France 1992), we were concerned that samples from different months might give different nutrient–response relationships. Specifically, we were curious whether LaBaugh's (1979) contention, based on data from a single lake, that the late summer period came closest to approximating CHL*a* and TP values for

Received June 25, 1993. Accepted March 7, 1995.
J11985

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Table 1. Regression statistics for relationships (Fig. 1) predicting seasonal mean log CHL*a* from monthly measurements of log CHL*a* in north-temperate lakes.

Month	Slope		Intercept	R^2	Sy:*x*	*N*
	Model I	Model II				
April	0.60	0.76	0.52	0.63	0.30	168
May	0.71	0.89	0.39	0.64	0.30	221
June	0.78	0.98	0.34	0.64	0.30	240
July	0.79	0.87	0.25	0.82	0.21	252
August	0.78	0.86	0.24	0.82	0.21	250
September	0.80	0.87	0.17	0.84	0.20	232
October	0.84	0.99	0.17	0.72	0.27	204

Note: R^2, coefficient of determination; Sy:*x*, standard error of the estimate; and *N*, number of lake-years.

Table 2. Regression statistics for relationships (Fig. 3) predicting seasonal mean log TP from monthly measurements of log TP in north-temperate lakes.

Month	Slope		Intercept	R^2	Sy:*x*	*N*
	Model I	Model II				
April	0.74	0.81	0.42	0.83	0.16	43
May	0.81	0.92	0.33	0.77	0.19	64
June	0.85	0.94	0.23	0.81	0.18	64
July	0.92	0.95	0.13	0.94	0.10	69
August	0.85	0.91	0.29	0.88	0.14	63
September	0.84	0.92	0.26	0.84	0.16	54
October	0.91	0.98	0.14	0.86	0.16	38

Note: Abbreviations are identified in Table 1.

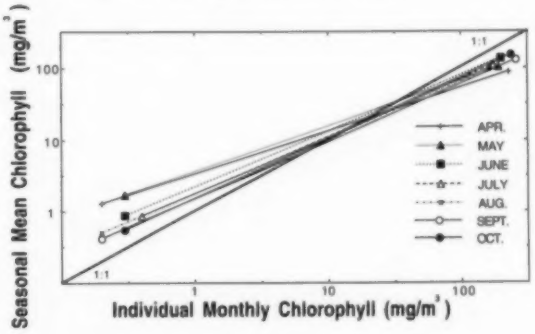
the entire season, holds generally. We therefore assembled monthly estimates of TP and CHL*a* collected during the growing season from a wide selection of north-temperate lakes. These data were used to determine which monthly (April to October) samples best approximated mean TP and mean CHL*a*, and which best predicted the relationship between the two.

Methods

Time series showing the phenological development of epilimnetic CHL*a* and TP were obtained for North American and European lakes listed in Marshall et al. (1988), France and Peters (1992), and France et al. (1994). Data presented in figures were read with a HIPADc digitizing pad (Houston Instruments, Austin, Texas). From four to seven TP and CHL*a* values were determined for each lake-year's time series at approximately mid-monthly intervals from April through October. This ensured that a standardized sampling program of similar replication and date was applied to all lakes (see France and Peters 1992). Individual CHL*a* values ranged from 1 to 175 $mg \cdot m^{-3}$, and individual TP values ranged from 2 to 250 $mg \cdot m^{-3}$. All analyses were based on log values to normalize the data as per convention (cf. Marshall et al. 1988).

Our analyses were concerned with how similar or dissimilar monthly values were from their seasonal means. To this end, we regressed the seasonal mean values against

Fig. 1. Relationships predicting seasonal mean CHL*a* from monthly CHL*a* in north-temperate lakes. Model I regression statistics are presented in Table 1.



individual monthly estimates. The best estimate should yield a regression with a slope (either model I or model II, Ricker 1973) close to unity, an intercept close to zero, a coefficient of determination (R^2) close to one, and a standard error of the estimate (Sy:*x*) close to zero.

Results and discussion

Inaccuracies (i.e., deviations between monthly and seasonal mean values) for CHL*a* are greater when single samples

Fig. 2. Scatterplots between April or September CHL*a* and seasonal mean CHL*a* in north-temperate lakes. Model I regression lines from Table 1 are indicated.

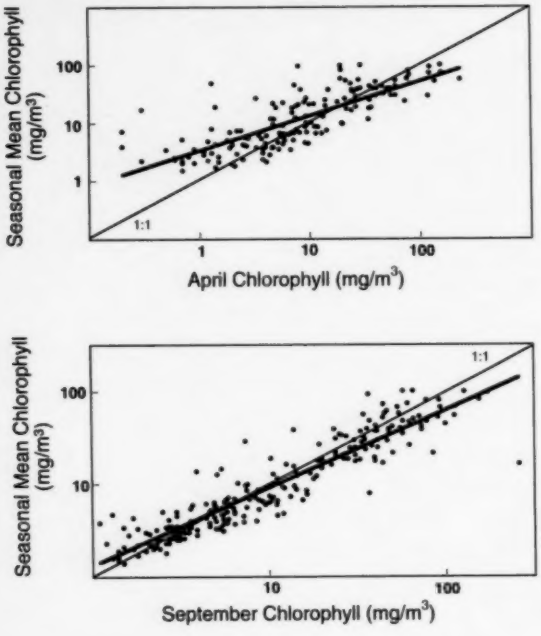


Fig. 3. Relationships predicting seasonal mean TP from monthly TP in north-temperate lakes. Model I regression statistics are presented in Table 2.

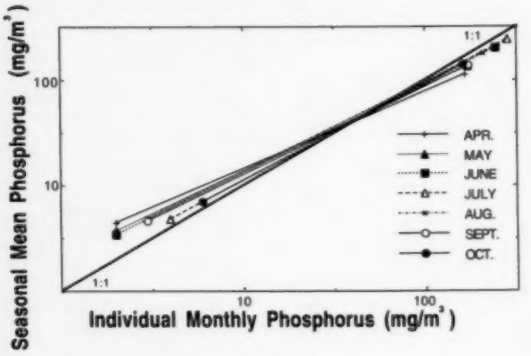


Fig. 4. Scatterplots between May or July TP and seasonal mean TP in north-temperate lakes. Model I regression lines from Table 2 are indicated.

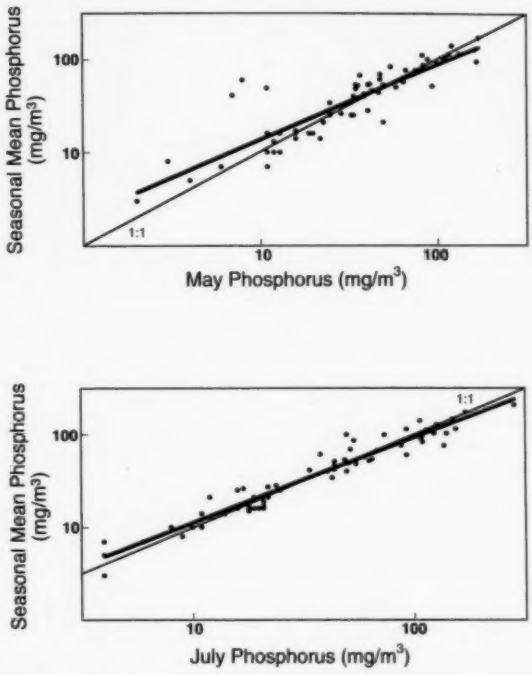


Table 3. Model I regression statistics for relationships predicting log CHL*a* from log TP in north-temperate lakes on the basis of monthly samples as well as seasonal mean values.

Relationship	Slope	Intercept	R^2	Sy:x	N
Mean	0.94	−0.39	0.68	0.27	74
April	0.60	0.09	0.33	0.42	43
May	0.78	−0.23	0.38	0.42	66
June	0.67	−0.15	0.31	0.45	67
July	0.84	−0.27	0.49	0.39	71
August	0.99	−0.51	0.48	0.47	60
September	0.96	−0.35	0.56	0.36	54
October	0.77	−0.06	0.48	0.37	46

Note: Abbreviations are identified in Table 1.

are taken in either April or May but reduced when single sampling is conducted in either September or October (Table 1, Fig. 1). The month:mean regression equation was found to be least precise (based on R^2 and Sy:x values) for April and most precise for July to September (Table 1, Fig. 2). If estimates of mean CHL*a* are to be made based on samples collected during single visits to a group of lakes, then the optimal time for the visit is from mid-July to mid-September.

For regressions between monthly and seasonal mean TP, July and October values were closest to the means, while, as in the case for CHL*a*, April values departed most from the 1:1 relationship (Table 2, Fig. 3). The month:mean regression equation for TP was the least precise for May and the most precise for July (Table 2, Fig. 4). Overall, monthly values more closely approximated the seasonal mean for TP than for CHL*a*. This was expected as TP is less variable about its individual mean than is CHL*a* (France and Peters 1992). If seasonal mean TP is to be estimated from single sampling visits, then July is the optimal month in which to sample, although any time thereafter is reasonably effective.

Table 4. Model I regression statistics for relationships predicting log mean CHL*a* from log April, log spring (April or May), or log mean TP in north-temperate lakes.

Relationship	Slope	Intercept	R^2	Sy:x	N
April TP : Mean CHL*a*	0.78	−0.14	0.50	0.35	39
Mean TP : Mean CHL*a*	1.07	−0.64	0.68	0.28	39
Spring TP : Mean CHL*a*	0.67	0.03	0.40	0.37	64
Mean TP : Mean CHL*a*	0.98	−0.49	0.64	0.29	64

Note: Abbreviations are identified in Table 1.

The TP:CHL*a* relationships developed from samples taken in April or June show the least predictive power, while that for September explains the most variability in CHL*a* (Table 3). If only a single visit is possible during a survey to estimate the nutrient–response relationship for a group of lakes, April is the worst month to sample, while September is the optimal time to sample. Our results therefore support LaBaugh's (1979) contention that data from the late summer best approximate CHL*a* and TP values for the entire growing season. In their early use, nutrient–response models were based on TP values collected in spring (Dillon and Rigler 1974) under the assumption that spring overturn averaged lake conditions. The present analyses indicate that regressions of mean CHL*a* to mean TP had more predictive power then did regressions of mean CHL*a* to either April TP or to April–May (i.e., spring) TP (Table 4). This occurs because the variability in TP is greatest during the spring.

Smith and Shapiro (1981) showed that seasonal variability in CHL*a* may be large enough to obscure nutrient responses that become apparent only when data are averaged over wider time intervals. In the present study, we found that the variance explained by regressions of monthly CHL*a* against monthly TP was substantially lower than that explained by the regression of mean CHL*a* against mean TP (Table 3). The loss of explained CHL*a* variation ranged from as low as 12% for September data to as high as 35% for April data. Whether such a loss is acceptable depends on the study objectives. Therefore, decreasing temporal replication to increase the number of lakes surveyed is a balancing strategy that sacrifices predictive power. The present results demonstrate, however, that this error in synoptic surveys can be reduced if sampling is limited to late summer or early autumn.

Acknowledgements

This work was supported by grants from the Government of Canada through the Natural Sciences and Engineering Research Council and the Government of Quebec through Fonds pour la formation de chercheurs et l'aide à la recherche, and is a contribution to the Limnology Research Centre of McGill University and the Group for Interuniversity Research in Limnology.

References

Dillon, P.J., and F.H. Rigler. 1974. The phosphorus–chlorophyll relationship in lakes. Limnol. Oceanogr. 19: 767–773.

El-Shaarawi, A.H., and R.E. Kwiatkowski. 1977. A model to describe the inherent spatial and temporal variability of parameters in Lake Ontario. J. Great Lakes Res. 3: 177–183.

Environmental Protection Agency. 1978. Summary analysis of the North American (U.S. portion) OECD eutrophication project: nutrient loading, lake response relationships and trophic state indices. U.S. Environ. Prot. Agency Ecol. Res. Ser. No. 3-78-008.

France, R.L. 1992. Climatic governance of the latitudinal trend in seasonality of freshwater phytoplankton production. Int. J. Biometeorol. 36: 243–244.

France, R.L., and R.H. Peters. 1992. Temporal variance function for total phosphorus concentration. Can. J. Fish. Aquat. Sci. 49: 975–977.

France, R.L., R.H. Peters, and Y. Prairie. 1994. Examination of temporal weighting as a seasonality correction for phosphorus–chlorophyll response models. Aquat. Sci. 56: 106–114.

Hanna, M., and R.H. Peters. 1991. Effect of sampling protocol on estimates of phosphorus and chlorophyll concentrations in lakes of low to moderate trophic status. Can. J. Fish. Aquat. Sci. 48: 1979–1986.

Heyman, U., S. Ryding, and C. Forsberg. 1984. Frequency distributions of water quality variables. Relationships between mean and maximum values. Water Res. 18: 787–794.

Knowlton, M.F., M.V. Hoyer, and J.R. Jones. 1984. Sources of variability in phosphorus and chlorophyll and their effects on use of lake survey data. Water Resour. Bull. 20: 397–407.

Kwiatkowski, R.E. 1985. Importance of temporal variability to the design of large lake water quality networks. J. Great Lakes Res. 11: 462–477.

LaBaugh, J.W. 1979. Chlorophyll prediction models and changes in assimilation numbers in Spruce Knob Lake, West Virginia. Arch. Hydrobiol. 87: 178–197.

Marshall, C.T., A. Morin, and R.H. Peters. 1988. Estimates of mean chlorophyll-*a* concentration: precision, accuracy, and sampling design. Water Resour. Bull. 24: 1027–1034.

Paulsen, S.G. 1992. EMAP-surface waters. What is it and what will it provide? Lake Line, 12: 25–31.

Ricker, W.E. 1973. Linear regression in fishery research. J. Fish. Res. Board Can. 30: 409–434.

Smith, V.H., and J. Shapiro. 1981. Chlorophyll–phosphorus relations in individual lakes: their importance to lake restoration strategies. Environ. Sci. Technol. 15: 444–452.

Biogeochemical origin of $\delta^{34}S$ isotopic signatures in a prairie marsh

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Abstract: Sulfur isotope ratios were determined in a prairie marsh at Delta, Manitoba, Canada, to determine whether organic matter produced in the marsh had distinctive isotopic signatures. High sulfate levels in sediments from the Delta Marsh arise from high salt concentrations in soils. Sulfate reduction is the major anaerobic metabolic pathway in this sediment system and sulfate diffused into sediment both from deeper soil horizons and from overlying water. Surface water $\delta^{34}S\text{-}SO_4^{2-}$ values of 15 ± 1‰ increased to a maximum of almost 40‰ in deeper sediment horizons, in marked contrast to sediment pore water $\delta^{34}S\text{-}H_2S$ values of 2 ± 4‰. The high degree of discrimination between $\delta^{34}S$ in sulfate and in hydrogen sulfide is similar to that found in brackish and marine ecosystems. Emergent macrophytes such as phragmites and cattail had isotopic signatures similar to reduced sulfur in this system. The data from this study suggest that in some nontidal wetland ecosystems, sulfur isotopes may be useful in determining producer–consumer relationships in a manner similar to their application in marine ecosystems.

Résumé : On a calculé les rapports isotopiques du soufre dans un marais de prairie à Delta, au Manitoba (Canada), pour déterminer si la matière organique produite y présentait des signatures isotopiques particulières. Les teneurs élevées en sulfate observées dans les sédiments provenant du marais de Delta sont liées aux fortes concentrations de sel dans les sols. La réduction des sulfates constitue la principale voie métabolique anaérobie dans ce système de sédiments et le sulfate se répand dans les sédiments depuis les horizons pédologiques inférieurs et depuis l'eau surnageante. Les valeurs du $\delta^{34}S\text{-}SO_4^{2-}$ des eaux superficielles sont passées de 15 ± 1‰ à un maximum de près de 40‰ dans les horizons de sédiments plus profonds, alors que la valeur de $\delta^{34}S\text{-}H_2S$ dans l'eau interstitielle des sédiments n'est que de 2 ± 4‰. La forte différence observée entre le $\delta^{34}S$ des sulfates et celui du sulfure d'hydrogène est semblable à celle que l'on observe entre des écosystèmes saumâtres et des milieux marins. Des macrophytes émergés, comme des roseaux et des quenouilles, présentaient des signatures isotopiques semblables à celles du soufre réduit dans ce système. Les données obtenues semblent indiquer que, dans certains écosystèmes à milieux humides non soumis à la marée, les isotopes du soufre peuvent permettre de déterminer les rapports producteur–consommateur d'une façon semblable à celle dont on les utilise dans les écosystèmes marins.

[Traduit par la Rédaction]

Introduction

The anaerobic decomposition of organic matter in freshwater ecosystems is predominantly through methanogenesis, while in marine systems, high concentrations of dissolved sulfate generally result in sulfate reduction as the main pathway for anaerobic metabolism (Capone and Kiene 1988). During dissimilatory sulfate reduction, there is a change in the isotopic composition of sulfur, with a lower $^{34}S/^{32}S$ ratio in sulfide relative to the original sulfate (Goldhaber and Kaplan 1974; Chanton et al. 1987). At the low sulfate concentrations typical of most freshwater lakes and marshes, sulfur isotopic fractionation is much lower than in marine sediments (Nriagu and Soon 1985; Fry 1986*a*, 1986*b*). However, inland aquatic ecosystems with high sulfate concentrations are found in many locations in North America (Bierhuizen and Prepas 1985; LaBaugh 1989). In such systems, sulfate reduction can be more important than methanogenesis to sediment anaerobic metabolism and the isotopic fractionation of sulfate during dissimilatory sulfate reduction is similar to that in marine sediments (Komor 1992).

In salt marsh ecosystems, vascular plants apparently derive part of their sulfur nutrition from isotopically light sulfide (Carlson and Forrest 1982). In general, algae derive their sulfur from isotopically heavier water-column sulfate (Trust and Fry 1992). Sulfur isotopes, often in combination with carbon and nitrogen stable isotopes, are thus useful for discriminating algal and vascular plant sources of organic

Received October 21, 1994. Accepted March 2, 1995.
J12612

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matter in salt marsh food webs (Peterson et al. 1985; Sullivan and Moncreiff 1990). Sulfur isotopic signals have not been used for discerning producer–consumer relationships in inland ecosystems, largely because there has been no expectation that this approach would be useful.

The Delta Marsh in central Manitoba has relatively high sulfate concentrations (5.5 mmol·L^{-1}) in surface water (Kadlec 1986*a*) and provides an excellent site for the examination of prairie marsh sulfur dynamics. In this paper we use measurements of (*i*) pore water dissolved sulfate concentrations, sulfide concentrations, and $\delta^{34}S$ ratios, (*ii*) sediment sulfur isotopic composition, and (*iii*) $\delta^{34}S$ values of several plant species to examine the biogeochemistry of sulfur isotopes in the Delta marsh. Two questions are addressed by this research: (*i*) is there a large sulfur isotopic fractionation between sulfate and hydrogen sulfide produced by dissimilatory sulfate reduction in high sulfate inland marshes; and (*ii*) do emergent macrophytes such as cattail (*Typha* spp.) have isotopic signatures that indicate the uptake of isotopically light reduced S? We have previously demonstrated the use of a multiple stable isotope approach for determining producer–consumer relationships in this system (Neill and Cornwell 1992). Although sulfur stable isotopes are useful in studies of S sources, transformation, and fate in aquatic sediments (Goldhaber and Kaplan 1974; Jorgensen 1979; Nriagu and Soon 1985; Chanton et al. 1987), in this study we explore the relationship between pore water chemistry and plant composition.

Methods

Plants, sediment pore waters, and sediment solid phases were collected from the Marsh Ecology Research Complex in the Delta Marsh (Batt et al. 1982), a 20 000 ha lacustrine marsh, in south-central Manitoba, Canada (50°11′N, 98°19′W). The study area was maintained at natural water levels in 1980, deeply flooded to eliminate emergent vegetation in 1981 and 1982 (Batt et al. 1982), drained to encourage recolonization by emergent vegetation in 1983 and 1984, and reflooded to average or slightly above average water levels in 1985 to 1988 . Marsh emergent vegetation consisted of cattail (primarily *Typha glauca*), common reed (*Phragmites australis*), bulrushes (*Scirpus acutus*, *Scirpus validus*), and whitetop (*Scolochloa festucacea*). Submerged vegetation consisted of sago pondweed (*Potamogeton pectinatus*) and bladderworts (*Utricularia* spp.). Metaphyton composed of *Chaetophora* spp. and *Cladophora* spp. dominated open water areas. Organic matter concentrations in surficial sediments in the experimental cells ranged from 20 to 70%; macrophyte detritus was evident on all surficial sediments. Low dissolved oxygen concentrations were only found in sites with emergent vegetation.

Hesslein (1976) pore water samplers were deployed for 10 days in July and September 1988. In July, two samplers were deployed in sediments with an overlying water depth of about 50 cm, at a site dominated by metaphyton (site A). One sampler was used for sampling pore water concentrations of SO_4^{2-}, ΣH_2S, and NH_4^+ while the other was used to analyze the isotopic ratios of SO_4^{2-} and ΣH_2S. To obtain sufficient sample for isotopic analysis, we combined all of the sample ports from 1 to 7, from 12 to 18, and from 24 to 30 cm to obtain about 60 mL of sample for each interval. In September 1988, 15 samples were collected for SO_4^{2-} analysis at site A and 11 were collected at a 10 cm deep site in a cattail (*T. glauca*) stand (site B).

All pore water samples were syringe filtered with Gelman GF/F glass fiber filters. Samples for ΣH_2S analysis were analyzed colorimetrically following Parsons et al. (1984). The samples for pore water SO_4^{2-} and Cl^- analyses were purged with N_2 to minimize the oxidation of ΣH_2S to SO_4^{2-} and refrigerated until analysis. A Dionex ion chromatograph was used for SO_4^{2-} analysis. For the S isotope samples, the pore water ΣH_2S was acidified and purged with N_2 into a zinc acetate trap. The zinc sulfide was converted to silver sulfide by the addition of silver nitrate following Hall et al. (1988). Barium chloride was added to the acidified pore water sample and filtered surface water samples and the resultant barium sulfate precipitate was collected on Gelman GF/F filters for isotopic analysis. Plant samples were collected from representative stands of cattail, phragmites, pondweed, and metaphyton. The preparation and isotopic analysis of plant samples are described in Neill and Cornwell (1992). Sulfur isotopic analyses were carried out by Coastal Science Laboratories, Austin, Texas. All S isotope data are expressed in standard δ notation relative to the Canyon Diablo troilite standard.

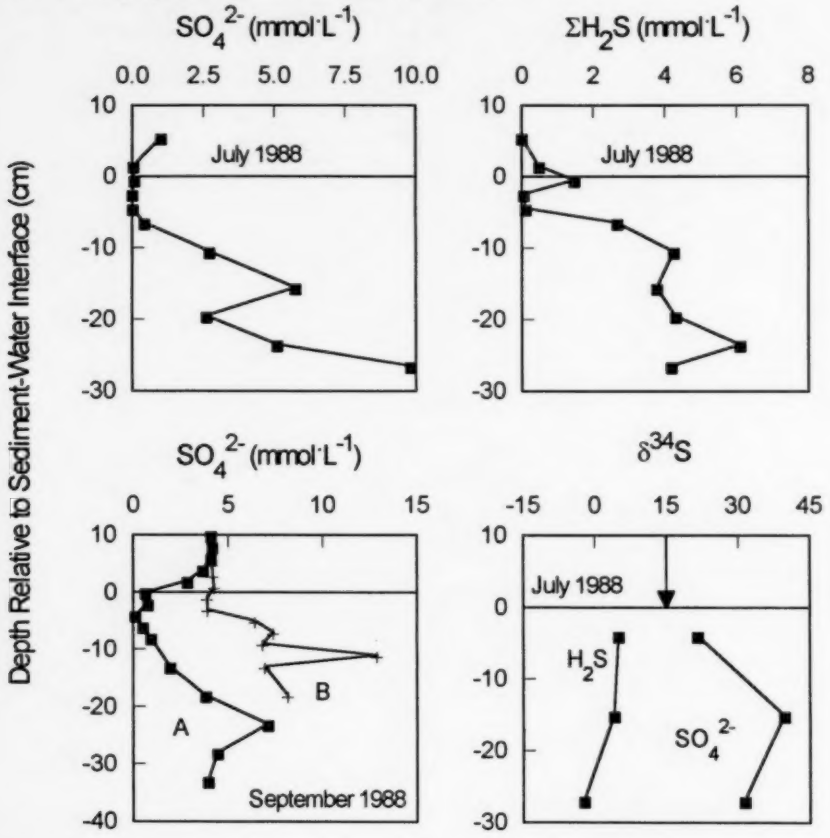
Results

At the open water site (A), a subsurface minimum in sulfate concentration was observed at 2–4 cm depth, with minimum concentrations of 0.01 in July and 0.1 mmol·L^{-1} in September (Fig. 1). In July, the maximum pore water sulfate value of 9.8 mmol·L^{-1} was considerably higher than average surface water values of ~5.5 mmol·L^{-1} (Kadlec 1986*b*). The shape of the sulfate profile in September resembled that in July, although surface water concentrations were higher and pore water concentrations were lower in the later sampling. The cattail site (B) had high sulfate concentrations in deeper sediment horizons, with virtually no distinct subsurface depletion at 0–4 cm depth. The pore water ΣH_2S concentrations during July were low immediately above the sediment–water interface, low at the 2–3 and 4–5 cm sediment horizons, and very high at 7 cm and deeper (Fig. 1).

The $\delta^{34}S$-SO_4^{2-} values for surface waters were very similar for three different water samples and averaged 15.0 ± 0.9‰ (Table 1). The sediment $\delta^{34}S$-SO_4^{2-} was enriched in ^{34}S relative to SO_4^{2-} in surface water. Pore water $\delta^{34}S$-ΣH_2S values were considerably lower than $\delta^{34}S$-SO_4^{2-} values. The difference between pore water $\delta^{34}S$-SO_4^{2-} and $\delta^{34}S$-ΣH_2S values ranged from 16.5‰ in the 1–7 cm horizon to 25.7‰ at 12–18 cm. Sediment $\delta^{34}S$ values were slightly lower than the values found for deep pore water $\delta^{34}S$-ΣH_2S.

Typha glauca and *Phragmites australis* had $\delta^{34}S$ values closer to that of sulfide than to either surface water or pore water sulfate values, strongly suggesting that these plants derive part of their sulfur nutrition from reduced sulfur. Both *Chaetophora* spp. and *Potamogeton pectinatus* had $\delta^{34}S$ values closer to that of surface water sulfate than to that of reduced S, suggesting that sulfate is their main source of sulfur nutrition.

Fig. 1. Vertical profiles of pore water sulfur sulfate, sulfide, and $\delta^{34}S$ in experimental impoundments at the Delta Marsh Ecology Research Complex. Metaphyton-dominated site A (■) was 50 cm deep and was sampled in July and September. Site B, located in a cattail stand with 10 cm of overlying water, was sampled only in September (+). The $\delta^{34}S$ of overlying water sulfate is indicated by an arrow.



Discussion

The presence of high concentrations of salts in northern Great Plains soils (Last 1989) contributes to high sulfate concentrations in surface and pore waters in the northern Great Plains region. Sulfate is the most abundant anion in the surface water of the majority of lakes and wetlands in Saskatchewan, Manitoba, North Dakota, and South Dakota, and water bodies with high surface water sulfate concentrations (>5 mmol·L^{-1}) are common (Labaugh 1989). The surface and pore water sulfate concentrations we measured in the Delta Marsh are probably typical of sulfate-dominated wetlands in the region. The average composition of surface waters in this system (Kadlec 1986*a*) is equivalent to 9% of seawater salinity and 18% of seawater sulfate concentration.

The high sediment ΣH_2S concentrations in Delta Marsh sediments suggest very high rates of sulfate reduction. Because of sulfate replenishment from surface and deep pore waters, sulfate reduction appears to be the predominant anaerobic metabolic pathway. The pore water sulfate and ΣH_2S profiles are very similar in shape to those measured in Devils Lake, North Dakota (Komor 1992). In that study, sulfate reduction in near-surface sediments was supported by diffusion of sulfate from both surface waters and deep pore waters. In the Delta Marsh, measurable ΣH_2S concentrations at 1.5 cm above the sediment–water interface and a high concentration of 1.47 mmol·L^{-1} in the top 1 cm of sediment are probably a result of the decomposition of metaphyton that settled to the sediment–water interface during the course of equilibrator deployment.

As in marine and estuarine sediments, dissimilatory sulfate reduction results in the production of reduced sulfur that is isotopically light relative to sulfate. The fractionation of 16.5 to 25.7‰ is at the low end for marine ecosystems (Goldhaber and Kaplan 1974; Chanton et al. 1987), but higher than that typical for low sulfate lake sediments (Nriagu and Soon 1985; Fry 1986*a*, 1986*b*). The almost total depletion of sulfate in near-surface sediment horizons at site A may limit the degree of isotopic fractionation compared with systems in which the degree of sulfate depletion is low (Jorgensen 1979). The relatively large differences in $\delta^{34}S$-SO_4^{2-} values between surface and pore

Table 1. Sulfur isotopic data.

	Site	Horizon (cm)	$\delta^{34}S$ (‰)
Surface water SO_4^{2-}	A		15.6
	A		14.0
	A		15.4
Pore water SO_4^{2-}	A	1–7	21.6
	A	12–18	39.7
	A	24–30	31.4
Pore water H_2S	A	1–7	5.1
	A	12–18	4.2
	A	24–30	−2.1, −2.2
Sediment	A		−4.5
	A		−4.5
	B		−5.5
Primary producers			
Typha glauca	A		4.1
Typha glauca	B		−5.7, −5.3
Phragmites australis	A		−3.8
Chaetophera sp.	A		18.3
Potamogeton pectinatus	A		12.3

Note: Where two values are shown, they represent replicated sulfur isotope analyses.

waters may result from differing SO_4^{2-} sources, with surface waters having inputs from the atmosphere or exchange with Lake Manitoba. The reoxidation of isotopically light pore water ΣH_2S would make the surface water $\delta^{34}S$-SO_4^{2-} lighter than the original sulfate. The $\delta^{34}S$ values of the pore water inorganic S (SO_4^{2-} + ΣH_2S), calculated from the concentrations and the isotopic composition of SO_4^{2-} and H_2S, were 6.5‰ at 1–7 cm, 15.3‰ at 12–18 cm, and 22.4‰ at 24–30 cm. These values span the values for surface water SO_4^{2-} and suggest that sediment-derived fluxes of sulfur could strongly influence the isotopic composition of surface waters.

The distinct isotopic signatures of surface water sulfate and pore water sulfide indicate that the natural abundance of sulfur isotopes can be useful for determining producer–consumer relationships in a manner similar to their application in marine ecosystems. Plant $\delta^{34}S$ values are generally 0–2‰ lighter than the sulfate they utilize (Trust and Fry 1992). Both cattail and phragmites had $\delta^{34}S$ values considerably lighter than sulfate and in the range of pore water sulfide. These $\delta^{34}S$ values fell within the range of values observed for salt marsh plants (Peterson and Fry 1987) and it appears that uptake of reduced sulfur by cattail and phragmites is similar to that of *Spartina alterniflora* in estuarine ecosystems. The $\delta^{34}S$ values of *Chaetophora* spp. and *Potamogeton pectinatus* most closely reflected surface water sulfate. Phytoplankton and periphyton were not analyzed for isotopic composition because water samples contained both algae and detritus. On the basis of a 0–2‰ depletion of plant $\delta^{34}S$ relative to a surface water $\delta^{34}S$-SO_4^{2-} value of 15‰, we expect algae in the Delta Marsh to have $\delta^{34}S$ values of 13–15‰, in the same general range found for *Chaetophora* spp. and *Potamogeton pectinatus*.

Although we made relatively few measurements of sediment and plant $\delta^{34}S$ in this study, the broad range of values for different producer groups suggests that sulfur isotopes can be useful for food chain studies in marshes that have high sulfate concentrations. In such marsh ecosystems, isotopic mass balances of sulfur can help to elucidate pathways of sulfur transformation. Further sulfur isotope analyses in other inland marsh systems, including those with lower sulfate concentrations, are required to determine whether the production of isotopically light sulfide by dissimilatory sulfate reduction results in isotopic signals that are useful for biogeochemical studies of sulfur in other nontidal marshes.

Acknowledgments

This study was funded by the Delta Waterfowl and Wetlands Research Station, Portage La Prairie, Manitoba. We thank Mike Owens for analytical assistance and Judith Stribling for valuable discussions.

References

Batt, B.D., P.J. Caldwell, C.B. Davis, J.A. Kadlec, R.M. Kaminski, H.R. Murkin, and A.G. van der Valk. 1982. The Delta Waterfowl Research Station – Ducks Unlimited (Canada) Marsh Ecology Research Program. *In* First Western Hemisphere Waterfowl and Waterbird Symposium, Edmonton, Alta., 28–28 May, 1982. *Edited by* H. Boyd. Canadian Wildlife Service, Ottawa, Ont. pp. 19–23.

Bierhuizen, J.F.H., and E.E. Prepas. 1985. Relationship between nutrients, dominant ions, and phytoplankton standing crop in prairie saline lakes. Can. J. Fish. Aquat. Sci. 42: 1588–1594.

Capone, D.G., and R.P. Kiene. 1988. Comparison of microbial dynamics in freshwater and marine environments: contrasts in anaerobic carbon metabolism. Limnol. Oceanogr. 33: 725–749.

Carlson, P.R., Jr., and J. Forrest. 1982. Uptake of dissolved sulfide by Spartina alterniflora: evidence from natural sulfur isotope abundance ratios. Science (Washington, D.C.), 216: 633–636.

Chanton, J.P., C.S. Martens, and M.B. Goldhaber. 1987. Biogeochemical cycling in an organic-rich coastal marine basin. 8. A sulfur isotopic budget balanced by differential diffusion across the sediment–water interface. Geochim. Cosmochim. Acta, 51: 1202–1208.

Fry, B. 1986*a*. Stable sulfur isotopic distributions and sulfate reduction in lake sediments of the Adirondack Mountains, New York. Biogeochemistry, 2: 329–343.

Fry, B. 1986*b*. Sources of carbon and sulfur nutrition for consumers in three meromictic lakes of New York State. Limnol. Oceanogr. 31: 79–88.

Goldhaber, M.B., and I.R. Kaplan. 1974. The sulfur cycle. *In* The sea. *Edited by* E.A. Goldberg. Wiley-Interscience, New York. pp. 569–655.

Hall, G.E.M., J.-C. Pelchat, and J. Loop. 1988. Separation and recovery of various sulphur species in sedimentary rocks for stable sulphur isotopic determination. Chem. Geol. 67: 35–45.

Hesslein, R.H. 1976. An in situ sampler for close interval pore water studies. Limnol. Oceanogr. 21: 912–914.

Jorgensen, B.B. 1979. A theoretical model of the stable isotope distribution in marine sediments. Geochim. Cosmochim. Acta, 43: 363–374.

Kadlec, J.A. 1986*a*. Effects of flooding on dissolved and suspended nutrients in small dike marshes. Can. J. Fish. Aquat. Sci. 43: 1999–2008.

Kadlec, J.A. 1986*b*. Input–output nutrient budgets for small diked marshes. Can. J. Fish. Aquat. Sci. 43: 2009–2016.

Komor, S.C. 1992. Bidirectional sulfate diffusion in saline-lake sediments: evidence from Devils Lake, northeast North Dakota. Geology, 20: 319–322.

LaBaugh, J.W. 1989. Chemical characteristics of water in northern prairie wetlands. *In* Northern prairie wetlands. *Edited by* A. van der Walk. Iowa State University, Ames, Iowa. pp. 56–90.

Last, W.M. 1989. Continental brines and evaporites of the northern Great Plains of Canada. Sediment. Geol. 64: 207–221.

Neill, C., and J.C. Cornwell. 1992. Stable carbon, nitrogen, and sulfur isotopes in a prairie marsh food web. Wetlands, 12: 217–224.

Nriagu, J.O., and Y.K. Soon. 1985. Distribution and isotopic composition of sulfur in lake sediments of northern Ontario. Geochim. Cosmochim. Acta, 49: 823–834.

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York.

Peterson, B.J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18: 293–320.

Peterson, B.J., R.W. Howarth, and R.H. Garritt. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science (Washington, D.C.), 227: 1361–1363.

Sullivan, M.J., and C.A. Moncreiff. 1990. Edaphic algae are an important component of salt marsh food-webs: evidence from multiple stable isotope analyses. Mar. Ecol. Prog. Ser. 62: 149–159.

Trust, B.A., and B. Fry. 1992. Stable sulphur isotopes in plants: a review. Plant Cell Environ. 15: 1105–1110.

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Canadian Journal of Botany / Revue canadienne de botanique	73	45	417§	162	417§	174	150
Canadian Journal of Chemistry / Revue canadienne de chimie	73	46	477	146	477	155	150
Canadian Journal of Earth Sciences / Revue canadienne des sciences de la Terre	32	47	398	128	398	134	150
Canadian Journal of Fisheries and Aquatic Sciences / Journal canadien des sciences halieutiques et aquatiques	52	261	365	130	395	145	150
Canadian Journal of Forest Research / Revue canadienne de recherche forestière	25	54	366	119	366	123	75
Canadian Journal of Microbiology / Revue canadienne de microbiologie	41	48	279	110	279	115	75
Canadian Journal of Physics / Revue canadienne de physique	73	49	296	94	296	96	75
Canadian Journal of Physiology and Pharmacology / Revue canadienne de physiologie et pharmacologie	73	50	347	112	347	119	75
Canadian Journal of Zoology / Revue canadienne de zoologie	73	51	449	166	449	175	150
Bimonthly / Bimestrielle							
Canadian Journal of Civil Engineering / Revue canadienne de génie civil	22	52	287	92	287	97	75
Genome / Génome	38	70	262	108	262	116	75
Canadian Geotechnical Journal / Revue canadienne de géotechnique	32	53	226	88	226	95	50
Quarterly / Trimestrielle							
Environmental Reviews/ Dossiers environnement	3	244	163	57	163	63	25

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National Research Council Canada
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Canadian Journal of Fisheries and Aquatic Sciences

Volume 52, Number 8, August 1995

Journal canadien des sciences halieutiques et aquatiques

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Printed in Canada by / Imprimé au Canada par
University of Toronto Press, Inc.
Typesetting by / Typographie par
Aubut & Nadeau Services Inc.

0706-652X(199508)52:8;1-X